Université de Montréal

A Québec Mystery Unveiled: The Quest to Understand Hereditary Sensory and Autonomic Neuropathy Type 2

par

Tina Thomas M.D.

Département de physiologie

Faculté de médecine

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TABLE OF CONTENTS

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Ce mémoire intitulé:

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présenté par:

Tina Thomas M.D.

a été évalué par un jury composé des personnes suivantes:

Dr John Kalaska
président-rapporteur

Dr Bernard Brais
directeur de recherche

Dre Andrea Richter
membre du jury

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SUMMARY

The Hereditary Sensory and Autonomic Neuropathies (HSANs) are caused by a degeneration of sensory and autonomic neurons. This thesis documents the characterization of the largest cohort of type 2 HSAN (HSAN2) described to date.

The clinical goals of this study were to confirm the main disease characteristics, to demonstrate age of onset and to evaluate clinical progression over time. From a molecular standpoint, the aims were to exclude linkage of HSAN2 to other HSAN loci, to identify the specific mutations in this group, and to demonstrate a founder effect for HSAN2 in Québec.

Seventeen patients were recruited. History and clinical exam confirmed that all patients suffered from the same disorder. Levels of sensory deficits were compared along the age spectrum. Genealogies were compiled and blood samples collected. A linkage study was performed to exclude linkage to the other HSAN loci. *HSN* gene sequencing was performed to determine the responsible mutations. Carrier frequencies were estimated using census data and Hardy-Weinberg proportions.

Sixteen French-Canadian patients participated. Analysis of deficit levels in the first thirteen patients showed progression with age followed by stabilization in adulthood. Genealogic study confirmed an autosomal recessive mode of transmission. All patients were from southern Québec; with 75% from the Lanaudière region. Linkage to the other HSAN loci was excluded and linkage to the HSAN2 locus at 12p13.33 confirmed. Gene sequencing demonstrated two mutations. Estimated regional carrier frequencies were 1:116 and 1:260 for the major (*c.943C→T*) and minor (*c.918-919insA*) halotypes, respectively. These mutations are predicted to result in truncation of the HSN2 protein.

Two founder mutations cause HSAN2 in the Lanaudière region. The disease manifests in infancy and progresses until adulthood. Current pathophysiologic hypotheses for the HSANs

implicate defective neuronal vesicular transport; however, the precise function of the novel HSN2 protein remains to be elucidated.

Key words: autosomal recessive neuropathies, founder effect, hereditary sensory and autonomic neuropathies, HSAN2, *HSN2* gene, HSN protein, neurodegenerative diseases, neuronal death, peripheral neuropathy

RÉSUMÉ

Les névrites héréditaires sensitives et autonomiques (NHSA) sont causées par la dégénérescence de neurones sensitifs et autonomiques. Ce mémoire décrit la plus large cohorte de NHSA de type 2 (NHSA2) à date.

Les objectifs cliniques de cette étude étaient de confirmer les principales caractéristiques et d'évaluer l'âge du début ainsi que la progressivité de la maladie. Du point de vue moléculaire, les buts étaient d'exclure la liaison de la NHSA2 aux loci des autres NHSA, d'identifier les mutations responsables et de démontrer un effet fondateur au Québec.

Dix-sept patients ont été recrutés. L'histoire et l'examen clinique ont confirmé que tous souffraient de la même maladie. Les niveaux des déficits sensitifs ont été étudiés en corrélation avec l'âge. Des généalogies ont été compilées et des échantillons sanguins prélevés. Une étude de liaison génétique a été faite pour exclure la liaison de la NHSA2 aux loci des autres NHSA. Le gène *HSN2* a été séquencé pour déterminer les mutations responsables. Les fréquences régionales des allèles ont été estimées à l'aide de données de recensement et des proportions de Hardy-Weinberg.

Seize patients Canadiens Français ont participé à l'étude. L'analyse des niveaux de déficit a démontré une progression avec la croissance, suivie d'une stabilisation à l'âge adulte. Les études généalogiques ont confirmé un mode de transmission autosomique récessif. Tous les patients provenaient du sud du Québec; 75% de la région de Lanaudière. La liaison aux loci des autres NHSA a été exclue, et la liaison au locus de la NHSA2 à 12p13.33 confirmée. L'étude de séquençage a démontré deux mutations. Les taux estimés de porteurs étaient de 1:116 et de 1:260 pour l'haplotype majeur (*c.943C→T*) et mineur (*c.918-919insA*), respectivement. Il est probable que ces mutations conduisent à une troncature de la protéine HSN2.

Deux mutations fondatrices sont responsables de la NHSA2 dans la région de Lanaudière. Cette maladie se manifeste à la petite enfance et progresse jusqu'à l'âge adulte. Les plus récentes hypothèses concernant la physiopathologie des NHSA mettent en cause des anomalies du transport vésiculaire neuronal; cependant, la fonction précise de la protéine HSN2 demeure à élucider.

Mots clés : effet fondateur, gène *HSN2*, mort neuronale, maladies neurodégénératives, neuropathie périphérique, neuropathies autosomiques récessives, neuropathie héréditaire sensitive et autonomique, NHSA2, protéine HSN2

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

AR	autosomal recessive
ARSACS	Autosomal Recessive Spastic Ataxia of Charlevoix Saguenay
CGRP	calcitonin gene-related peptide
cM	centimorgan
DNA	deoxyribonucleic acid
DRG	dorsal root ganglion
EMG	electromyography
ERK	extracellular signal-regulated kinase
F	female
FrCan	French-Canadian
HMSN/ACC	Hereditary Motor and Sensory Neuropathy with Agenesis of the Corpus Callosum
HSAN	Hereditary Sensory and Autonomic Neuropathy
HSAN2	Hereditary Sensory and Autonomic Neuropathy, type 2
HSN	Hereditary Sensory Neuropathy
IKBKAP, IKAP	I kappa B kinase complex-associated protein
IKK	I kappa B kinase
M	male
MAPK	mitogen-activated protein kinase
mRNA	messenger ribonucleic acid
N	normal
N/A	not available
NGF	nerve growth factor
NHSA	neuropathie héréditaire sensitive et autonomique
NHSA2	neuropathie héréditaire sensitive et autonomique de type 2
No.	number
NT	neurotrophin
MIM	Mendelian Inheritance in Man
P13K	phosphatidylinositol 3 kinase
PCR	polymerase chain reaction
PLC	phospholipase C
PKC	protein kinase C
POSS	possible
PRKWNK1	protein kinase, kinase deficient 1
RNA	ribonucleic acid
rtPCR	reverse transcriptase-polymerase chain reaction
SNAP	sensory nerve action potential
SPT	serine palmitoyltransferase
SPTLC1	serine palmitoyltranferase long-chain base subunit 1
Trk	tropomyosin-related kinase
TrkA	tropomyosin-related kinase type A
↑	increased
↓	decreased
↓↓	markedly decreased
+	present/yes
–	absent/no
+/–	variable
?	uncertain/unknown

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PREFACE

This study was inspired by a single patient with Hereditary Sensory and Autonomic Neuropathy Type 2, who presented for evaluation at the neurogenetics clinic of the Notre-Dame Hospital of the Centre Hospitalier de l'Université de Montréal (CHUM) in 1999. His extensive neurological deficits and striking mutilating changes of the extremities, along with the tumultuous psychological journey he described, motivated a deeper look at this disease. A review of the literature demonstrated a remarkable preponderance of French-Canadian HSAN2 cases, which included the very family that had a pivotal role in the first definition of the entity [1, 2]. This led to a search for other cases in Québec, and thus was launched our quest to elucidate the basis of this devastating disorder.

INTRODUCTION

HISTORICAL BACKGROUND

The hereditary sensory and autonomic neuropathies (HSANs) are a group of disorders whose pathophysiology is attributed mainly to the degeneration of primary sensory and autonomic neurons, with relative sparing of motor neurons [3, 4].

There have been many published cases of hereditary sensory neuropathy since the early 1900s. The disorders were usually identified and named for the predominant symptom or complication, which was the striking mutilating, yet painless changes of the extremities. As a result of this generalization, several disorders were grouped together, despite heterogeneous features, such as the distribution of sensory deficit, and variable presence of lightning pains, dysautonomia or cranial nerve deficit. The patients were said to have "mutilating acropathy," "neuropathic acro-osteolysis," "painless whitlows," or "mal perforant du pied," among other picturesque eponyms [5]. Another example, "Morvan's syndrome," shared the clinical features of dissociated sensory loss and painless whitlows, but also many features that we now know are clearly not part of the HSAN phenotype [3, 6]. In retrospect, Morvan's cases were most likely suffering from syringomyelia [7]. These misclassifications will be discussed in further detail in the first chapter.

Definition of the HSAN2 Disease Entity

The hereditary sensory neuropathies (HSNs) were first systematically classified by Ohta and Dyck in 1973, who initially proposed four clinical subtypes, HSNs 1 to 4 [2]. Dyck suggested that this name be modified to HS*A*N (Hereditary Sensory *and Autonomic* Neuropathies) in 1984, in consideration of the variable autonomic manifestations present in these disorders. Moreover, he later added HSAN5 to the classification (Tables I and II, pp. 18-19) [5].

This thesis is centered on the study of HSAN type 2, which was first defined by Ohta in his seminal 1973 paper [2]. The pathophysiologic basis of this disease is thought to be a progressive degeneration of sensory neurons in the dorsal root ganglia. Affected patients usually present in childhood with painless fractures of the extremities or foot and hand ulcers. Eventually, parts of limbs are actually lost through repeated infection and necrosis, as well as surgical amputation [2].

The features established by Dyck for HSAN2 are:

1. Inheritance pattern compatible with autosomal recessive transmission.
2. Onset of illness in early childhood.
3. Presence of soft tissue infections of the extremities, as well as fractures and neurogenic arthropathy (progressive deformation of joints caused by lack of sensation, repeated trauma, and fractures).
4. Sensory deficit involving all modalities and affecting predominantly the distal lower and upper extremities.
5. Absent or diminished deep tendon reflexes.
6. Absent or severely diminished myelinated fibers as well as decreased unmyelinated fibers on sural nerve biopsy.
7. Absence of postural hypotension, sphincter disturbance or impotence.
8. Absent sensory nerve action potentials (SNAP) on electromyography (EMG).
9. Possible anhidrosis most marked in the distal extremities [3].

Other Subtypes of HSAN

HSAN1 is the most common of the HSANs [8, 9]. It has in common with HSAN2 a progressive and symmetric sensory loss implicating predominantly pain and temperature, as well as possible distal anhidrosis, but has additional features that are not part of the HSAN2 phenotype. These include: presentation in the second or third decade (it is the only HSAN that

does not present in infancy), lancinating pains, burning feet paresthesias and sensorineural hearing loss [8, 10, 11, 12, 13]. There is also usually eventual distal motor involvement with weakness and atrophy [12]. Autonomic involvement is minimal, and blood pressure and sphincter function are normal [8, 12]. There are loss of deep tendon reflexes and progressive development of trophic ulcers and acral injuries as in HSAN2. Intradermal injection of histamine phosphate in involved areas does not elicit a normal unmyelinated C-fiber-elicited axon flare response. This is also seen in HSANs 2, 3 and 4 (but not in mild cases of HSAN2 or in HSAN5, where there are little reduction of C-fibers) [8]. Nerve conduction studies show reduced or absent SNAP, usually with preserved motor conduction. Sural nerve biopsy demonstrates fiber loss, which may be most marked in small myelinated and unmyelinated fibers [8, 12, 13, 14]. Dorsal roots and posterior columns are diminished in size on pathologic examination [8].

HSAN3, also known as *Familial Dysautonomia* or *Riley-Day Syndrome*, is principally distinguished from the other HSANs by involvement of both sensory and motor nerves. This disorder is almost exclusively seen in patients of Jewish Ashkenazi descent [9, 15]. These individuals develop diffuse insensitivity to pain, but rarely have mutilations of the extremities, even though they may develop Charcot joints. Visceral pain sensation is normal. Other unique features include: neonatal presentation with feeding difficulties and failure to thrive (from poor oral coordination and hypotonia), decreased gustation (due to absence of fungiform papillae), alacrima and decreased corneal reflexes (which together may result in corneal abrasions, infections and subsequent scarring), abnormal body temperature and blood pressure maintenance, skin blotching, and hyperhidrosis (due to labile vasomotor and cardiovascular function) [8, 12, 13]. These patients are also supersensitive to cholinergic and adrenergic agents [8]. Motor neuron involvement is manifested by infantile hypotonia, gait abnormalities and a high incidence of juvenile scoliosis in this population (85% by age 10 years) [8]. As in other HSANs, deep tendon reflexes are decreased or absent. Nerve conduction studies show decreased SNAPs, as well as decreased CMAP amplitudes and conduction velocities [13]. Nerve biopsy

shows loss of both unmyelinated and myelinated fibers (particularly small ones), and also of neurons in the Gasserian, dorsal root and autonomic (especially the sympathetic) ganglia [8, 13, 16, 17, 18]. Dorsal column size is diminished in older patients [18]. In addition, blood vessels have sparse or absent sympathetic terminals, which likely contributes to the vasomotor instability [8, 17]. The disease is inevitably fatal, with 50% mortality before age 40, mostly from pulmonary or cardiac complications [19, 20].

HSAN4, also known as *Congenital Insensitivity to Pain with Anhidrosis*, is the second most common type of the HSANs [21]. It presents in young children with a profound insensitivity to pain, which, unlike HSAN2, can include the trigeminal territory and the visceral organs [8, 12, 13]. The severity of the nociceptive sensory loss favors auto-mutilatory behavior. Biting of the tongue after tooth development may lead to a bifid or absent tongue and missing lip edges [22]. Biting of the fingers results in ulceration or loss of fingertips [22]. Repeated trauma and unrecognized fractures lead to the development of osteomyelitis, as well as Charcot joints. Other specific abnormalities include: recurrent and potentially life-threatening fevers of noninfectious origin, severe anhidrosis involving the trunk and upper extremities, and learning disabilities or mental retardation [12, 23]. Up to 20% of patients die from hyperpyrexia. Motor function and emotional tearing are normal [12]. Deep tendon reflexes are intact. There is no axon flare response to histamine. Nerve conduction studies are often normal [12, 13]. These patients have diffuse loss of unmyelinated fibers on nerve biopsy. In addition, absence of Lissauer's tracts and reduced numbers of DRG small neurons have been noted [12, 13, 24]. The spinal tract of the trigeminal nerve is also reduced in size with loss of small fibers [22]. Decreased skin innervation and absence of innervation of eccrine sweat glands have also been demonstrated [8, 24].

HSAN5 is a rare disorder, with only a few cases described. It is clinically similar to HSAN4, with normal nerve conduction studies, but nerve biopsy shows loss of small

myelinated fibers with relative preservation of unmyelinated fibers [8, 12, 13]. Sweating is preserved, unlike in HSAN4, and mental retardation is not a part of the phenotype [25, 26].

This improved clinical comprehension of the separate disease entities has not only permitted more accurate diagnosis of affected individuals, but has also set the foundation for the study of the biological and genetic bases of these disorders.

GENETICS OF THE HEREDITARY SENSORY NEUROPATHIES

Hereditary sensory neuropathies are clearly a clinically heterogeneous group of disorders. The genetic basis of the HSANs has been studied, initially to establish the mode of transmission (table I, p. 18). Most recently, positional cloning strategies have been used to identify the chromosomal loci of the mutated genes as well as the specific mutated genes (table III, p. 20).

Modes of Transmission

The modes of transmission of the HSANs are heterogeneous (table I, p. 18). An article published by Kondo et al. in the *Archives of Neurology* in 1974 analyzed the mode of transmission of the hereditary sensory neuropathies at large; that is, all types confounded in the same analysis [27]. They compared 66 families, dividing them into 42 "successive sibships," (families where one parent was affected by HSN), and 24 "nonsuccessive sibships," (where both parents were unaffected). There was a history of parental consanguinity in 83.3% of the nonsuccessive sibships (a frequent finding in recessively transmitted disorders), compared to none in the successive ones. In addition, using a statistical technique known as "segregation analysis of pooled pedigree data," they established "segregation ratios" (defined as the number of affected siblings divided by the total number of siblings) for the abnormal allele. The

segregation ratio was 0.26 in the nonsuccessive sibships, compared to 0.40 in the successive sibships. However, when females were excluded from the latter group, the segregation ratio rose to 0.50. These values are very compatible with autosomal recessive and dominant transmission, respectively. The lower value for females in the second group was attributed to incomplete penetrance in female heterozygotes. This mathematical analysis supported the genetic heterogeneity in HSANs that had been previously suggested in the literature [27].

In Donaghy's 1987 article published in *Brain*, the HSANs were classified into three groups based on mode of transmission [28]. HSAN1 has an autosomal dominant mode of transmission, while types 2, 3 and 4 are autosomal recessive. These authors also included X-linked forms of sensory neuropathy [28, 29]. HSAN5, which was not included in their analysis, has an autosomal recessive mode of transmission [9].

It has been confirmed by the kinships described in the literature that HSAN2 has an autosomal recessive pattern of transmission. For example, Ohta et al. described a Québec family, which included four affected siblings (three boys and one girl) out of six, while the parents and grandparents were asymptomatic. This is compatible with an autosomal recessive transmission of disease despite the presence of more than 25% of affected siblings [2]. This was the same family previously described by Dyck and by Hould in the 1960s [1, 30]. Other HSAN2 family trees described in the literature are also compatible with a recessive mode of transmission [31, 32, 33, 34]. In family "D" of Heller and Robb, the pedigree data presented is suggestive of an autosomal recessive transmission, though the authors initially proposed an autosomal dominant mode of transmission with reduced penetrance (Fig. 1, p. 21) [5, 32]. In fact, this family had four affected siblings out of seven (three boys and one girl), with unaffected parents and paternal grandparents, as well as two other affected relatives, who were nephews of the paternal great-grandmother. Upon analysis of the family tree, it is evident that both parents of the four affected children may have been carriers of a recessive gene, despite the higher than

expected transmission rate for an autosomal recessive disease. The gene from the paternal side may well also have been passed on to the two other relatives [35].

In conclusion, the variability, both in the clinical phenotype and in the genetic modes of transmission of the HSANs argued strongly in favor of distinct responsible mutations for these diseases.

Identified Chromosomal Loci and Gene Mutations for HSANs 1, 3, 4 and 5

HSAN1: 9q22.1-q22.3, SPTLC1 Mutations

The HSAN1 locus was discovered by Nicholson and colleagues in 1996 [10]. This group performed a genome scan with linkage analysis in four Australian families with HSAN1, and localized the gene to an eight-centimorgan (cM) region between markers D9S318 and D9S176 on chromosome 9q22.1-q22.3. Analysis of possible candidate genes demonstrated that the gene for the neurotrophic tyrosine kinase receptor type 2 mapped to chromosome 9q21-q22, but centromeric to the identified HSAN1 locus [10]. Subsequently, Blair et al. refined this interval to a three- to four-cM region between markers D9S1781 and FB19B7 [36]. Bejaoui et al. later confirmed the linkage of HSAN1 to chromosome 9q22 in a large American family of German origin, between markers D9S1797 and D9S197, in an estimated interval of 2.5 cM [37].

The *SPTLC1* (serine palmitoyltransferase, long-chain base subunit 1) gene was mapped to this locus in 2001 [38, 39]. All affected members in eleven families with HSAN1 had mutations in this gene. Three different missense mutations (398G→A, 399T→G and 431T→A) were identified in these families; two (*C133Y* and *C133W*) in exon 5 which resulted in cysteine→tyrosine and cysteine→tryptophan substitutions, respectively, and the other (*V144D*), located in exon 6, which resulted in a valine→aspartic acid substitution [38, 39]. It was hypothesized that these mutations could result in structural changes in the protein. The cysteine→tyrosine and cysteine→tryptophan mutations could do so by disruption of normal

disulfide bonding between cysteine residues. The valine→aspartic acid mutation causes substitution of a hydrophilic amino acid for a hydrophobic amino acid, thus likely leading to a conformational change [38]. The authors also demonstrated that these cases had increased *de novo* synthesis of glucosyl ceramide established from lymphoblast cell lines compared to healthy controls. Five cases had levels increased 175% compared to eight controls [38]. The *C133W* mutation was confirmed in a large cohort by Houlden et al. in 2006 [14].

Serine palmitoyltransferase (SPT) is a heterodimeric pyridoxal 5'-phosphate (PLP)-dependent enzyme composed of two subunits, long chain base subunits 1 (LCB1) and 2 (LCB2). The two subunits are encoded by the *SPTLC1* and *SPTLC2* genes, respectively. Both subunits are essential for SPT activity. SPT catalyzes the condensation of L-serine and palmitoyl coenzyme A to generate 3-ketodihydrosphingosine (KDS) in the first and rate-limiting step of sphingolipid synthesis. SPT is found in many types of tissues and is associated with the endoplasmic reticulum. Levels of SPT mRNA vary in different tissue and cell types and also in various developmental stages [40]. The amino acid sequences around Cys^{133} and Val^{144} of LCB1 are highly conserved in organisms from yeast to humans, suggesting that this domain has a critical function [40]. Tentative models of SPT tertiary structure show that both Cys^{133} and Val^{144} of LCB1 are close to the PLP-binding site of LCB2 [40]. The catalytic site of SPT is thought to be at the junction of the two subunits, with both contibuting. Sphingolipids are an important structural component of cell membranes in all tissues and are implicated in signal transduction and membrane trafficking pathways [9, 15, 40, 41, 42]. Sphingolipid intermediates include sphingoid bases and ceramide, which modulate cell proliferation, differentiation and apoptosis [40]. It has been shown that the production of ceramide by catabolism of sphingomyelin provokes cellular apoptosis, particularly of differentiating neurons, during the normal process of neural tube closure [43]. Dawkins et al. initially proposed that the mechanism of neuron loss in HSAN1 was increased enzymatic activity of the mutated SPT proteins ("gain-of-function mutations"), which led to accumulation of sphingolipid intermediates and resulted in ceramide-induced neuronal

death [38]. However, subsequent studies in cells, including cultured lymphoblasts from HSAN1 patients demonstrated that both mutations in fact *decreased* normal SPT activity and decreased the rate of *de novo* sphingolipid synthesis despite normal levels of the two individual subunits [12, 40, 44]. In experiments with cultured HSAN1 lymphoblasts, *C133Y* and *C133W* mutations did not alter levels of SPT subunits [40, 45]. In addition, studies of HSAN1-mutant cell lines showed that SPT complex formation was possible even with the LCB1 mutant protein [40]. Nevertheless, these mutations did result in reduced SPT activity and sphingolipid synthesis [45]. A study of transgenic mice with the *C133W* mutation demonstrated decreased SPT activity and development of age-dependent mild motor impairments [46].

All the preceding data have led to two general hypotheses to explain the pathophysiology of HSAN1. The "dominant negative effect" hypothesis suggests that peripheral nerves are sensitive to the metabolic repercussions of decreased substrate binding or catalysis by SPT [15]. An alternative "gain of function" theory proposes that the accumulation of the mutated LCB1 subunit in neurons may lead to toxic effects [15, 45]. It is not known why mutations in this ubiquitously-expressed protein cause pathological effects in only certain cell types within a tissue [15, 40, 45].

HSAN3: 9q31-q33, IKBKAP Mutations

HSAN3 was studied by Blumenfeld and colleagues [47]. In their 1993 linkage analysis of 26 families, they identified a candidate region for this recessive gene (named *DYS*) in an 11-cM region on chromosome 9q31-q33 flanked by markers D9S53 and D9S105 [47, 48]. They later narrowed the interval to 0.5 cM between markers 157A3 and 43B1GAGT through haplotype analysis [48]. In addition, this haplotype analysis showed that more than 98% of HSAN3 chromosomes in the study shared the same major (or more frequent) haplotype. In fact, *all* HSAN3 patients in this study had at least one copy of the major haplotype. This impressive linkage dysequilibrium indicates a common ancestry or "founder effect" for this disease. This

notion is reinforced by the observation that most cases of HSAN3 are of Jewish Ashkenazi descent. In order to establish the carrier frequency for the gene in this population, control chromosomes from spouses of carriers were analyzed. The major haplotype was present in only 1.54% of these chromosomes[48].

The group of Anderson and colleagues next studied the mRNAs encoded by this region in patients with the major and minor haplotypes. They noted that in cells of patients with the major haplotype, the RNA encoding the IKB kinase complex-associated protein (IKAP or IKBKAP; inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) was expressed, but the protein was truncated to 619 amino acids (as opposed to a normal 1332 amino acids) as a result of the skipping of exon 20 [49]. Sequence analysis of the IKAP-encoding gene in these patients revealed a T→C substitution (2507+6 T→C) in position 6 of the donor splice site of intron 20, which resulted in splicing out of both exon and intron 20 in IKAP mRNA. This produced the truncated IKAP protein. With the most common of the minor haplotypes, there was a missense mutation in exon 19 (2390 G→C) [50]. This resulted in both an arginine-to-proline substitution at amino acid residue 696 of IKAP (*R696P*) and the disruption of a consensus serine/threonine kinase phosphorylation site (RIVT→pIVT) [49]. This produced a full-length IKAP with a nonconsensus phosphorylation site. They confirmed this finding by immunoprecipitation of IKAP in [^{35}S]-methionine- or [^{32}P]-orthophosphate-labeled cells from a heterozygote for *R696P* and from a normal individual. Comparable levels of synthesis of IKAP were demonstrated, but with a reduced level of phosphorylation in cells with the *R696P* mutation [49]. These authors also studied the local expression of IKAP mRNA, and demonstrated the greatest levels in the cerebellum, thalamus, pituitary gland, and certain other regions of the brain. Expression in peripheral nerves was not studied. They suggested that the remarkable amounts of mRNA in cerebellar and thalamic tissue and the defective synthesis of normal IKAP protein in HSAN3 patients may link these regions to the pathophysiology of gait and coordination difficulties as well as of defective pain

and temperature sensation [49]. Cuajungco et al. studied the ratios of wild-type to mutant *IKAP* transcripts in cell lines, blood and postmortem tissues from HSAN3 patients with the major mutation [51]. They found wild-type *IKAP* transcripts in all of these, but to varying extents, indicating that the splice site mutation causes a decrease in splicing efficiency and decreased production rather than complete loss of the normal protein [15, 51]. Central and peripheral nervous tissues had the lowest expression levels of normal-length *IKAP* transcript [51]. This suggested that the more severe deficiency of correctly spliced *IKAP* transcript in the nervous tissues compared to other sites explains the selective neuronal maldevelopment and degeneration characteristic of this disease [20, 49, 51, 52].

The IKAP protein is presently believed to be a member of the *human holo-Elongator complex*, which is thought to facilitate transcription by RNA polymerase II and is required for the activation of many genes [20, 53, 54]. If this is the case, decreased amounts of normal IKAP in HSAN3 may prevent transcriptional events that are essential to normal neuronal development and function [55]. Elongator target genes were recently studied in fibroblast cell lines from HSAN3 patients and it was found that several code for proteins involved in cell motility [56]. Thus, deficiency of functional IKAP protein in neural tissues of HSAN3 patients may cause incomplete development and/or hinder the maintenance of sensory and autonomic neurons through altered cell motility and neurite outgrowth. Other hypotheses for the role of mutant IKAP protein include: loss of its ability to activate transcription factor NF-KB, loss of its regulatory function in c-*jun* N-terminal kinase (JNK) stress signaling (JNK has both protective and promotive roles in neuronal apoptosis), and loss of its activating role for regulators of cell-surface transport or exocytosis of post-Golgi secretory vesicles [9, 15, 50, 57, 58, 59].

HSAN4: 1q21- q22, TrkA Mutations

The mutation responsible for HSAN4 was not uncovered by a positional cloning approach as for HSAN types 1 and 3, but rather by a candidate gene strategy.

The expression of the TrkA (tropomyosin-related kinase type A) receptor during embryogenesis coincides with the timing of neural crest migration, spinal ganglion formation and morphogenesis of most cranial nerve ganglia [59]. In the study of Smeyne et al., knockout mice lacking the *Trk* proto-oncogene were demonstrated to have severe sensory and sympathetic neuropathies, with dramatic cell loss in trigeminal, sympathetic, and dorsal root ganglia, as well as in cholinergic basal forebrain projections to the hippocampus and to the cerebral cortex [60].

The TrkA receptor is a transmembrane tyrosine kinase receptor that is the high-affinity receptor for a member of the neurotrophin family known as *nerve growth factor* (NGF) [61]. In sensory neurons, TrkA is expressed in dorsal root ganglia (DRG) and other neural crest-derived ganglia [15, 62, 63]. In the DRG, the majority of NGF-responsive neurons are primary nociceptive C-fibers. In the central nervous system, TrkA expression is limited to basal ganglia and striatal cholinergic neurons [64]. Binding of NGF to the TrkA receptor results in receptor dimerization and autophosphorylation of tyrosine residues on the cytoplasmic tails of the receptors. These phosphorylated sites then become docking sites for intracellular proteins, and this initiates various cellular signal transduction cascades, including activation of the Ras/mitogen-activated protein kinase/extracellular signal-regulated kinase (Ras/MAPK/ERK), phosphatidylinositol 3 kinase/Akt (P13K/Akt) and phospholipase C/protein kinase C (PLC/PKC) pathways [8, 65, 66, 67]. The end results of these cascades influence transcription and promote neuronal differentiation and/or survival. Studies of tissue expression of the neurotrophins have shown their presence in sensory neurons themselves, as well as in peripheral nerve support cells (Schwann cells and fibroblasts). Furthermore, animal studies have demonstrated a differential pattern of expression during development, with up to 70% of DRG neurons expressing TrkA during rat embryogenesis compared to 40% in the adult [8]. Experiments have shown that 70-85% of DRG neurons require NGF for survival during embryonic development [68]. Many of these are small-diameter noiciceptive neurons and most express the TrkA receptor [68, 69]. These patterns of

expression suggest a role for the TrkA/NGF system in the development, regeneration, remodeling, and maintenance of these neurons [65, 68]. In fact, NGF is known to promote neurite outgrowth and survival of embryonic small-diameter sensory fibers and sympathetic neurons [8, 12, 15, 65, 70, 71, 72]. The *neurotrophic hypothesis* suggests that developing neurons must compete for neurotrophic factors produced by target tissues. Neurotrophins would function as survival signals and suppress apoptosis through local effects on axons as well as more remote nuclear effects on gene expression in the cell body [73]. Absence of neurotrophins would allow for activation of apoptotic cascades. Thus, only the neurons reaching their correct targets and obtaining sufficient neurotrophic factors survive [22]. This mechanism would account for accurate matching of the number and properties of innervating neurons to target tissue needs [22].

The gene for the TrkA/NGF receptor was localized on the long arm of chromosome 1 by two groups in 1990. Miozzo et al. applied *in situ* hybridization with a cDNA probe containing the whole *Trk* proto-oncogene to assign a localization at 1q32-q41 [74]. Morris et al. localized the gene to a more centromeric site at 1q23-q24 [75]. The precise structure of the gene was elucidated by two groups in 1996 [75, 77].

After the potential relationship between sensory and autonomic neuropathies and the *TrkA* gene was established in Smeyne's mouse model, Indo et al. studied NGF, p75 and TrkA as candidate genes and established that mutations in the *TrkA* gene were the basis for HSAN4 [61, 78]. This group studied the mRNA and genomic DNA of three unrelated cases of HSAN4 with consanguineous parents [78]. The first patient was homozygous, and her parents heterozygous, for a single base C deletion at nucleotide 1726 of exon C (1726-C). This deletion occurred in a region that encodes the tyrosine kinase domain and caused a frameshift and premature downstream termination codons. Sequencing of genomic DNA of the second patient showed that the 5' splice site of an intron between exons D and E contained an A→C substitution in the third position [78]. Similar mutations at this position had previously been shown to result in skipping of the preceding exon [79]. This proband, as well as his affected brother, were

homozygous for the substitution, while the parents were heterozygous [78]. The mutation was absent in the fifty controls studied. The third patient harbored a single-nucleotide substitution in exon C (1795 G→C), which resulted in a glycine-to-arginine substitution at amino acid 571. Gly 571 is located in the tyrosine kinase domain of the TrkA receptor and is conserved among fourteen receptor tyrosine kinases, which indicates that it is very likely important for their enzymatic activity. This patient was homozygous and his parents heterozygous for the missense mutation, which was again, not present in the fifty control patients [78].

In addition, Mardy et al. identified several different mutations of the *TrkA* gene in seven families with HSAN4 from five different countries in 1999 [80]. Yotsumoto et al. described a novel *TrkA* mutation in a large Japanese family with HSAN4 [81]. Greco et al. studied an Italian family with another novel *TrkA* mutation [82]. Shatzky et al. identified a 1926-ins-T mutation in the *TrkA* gene as responsible for the disease in Israeli-Bedouin Arabs [83]. Altogether, 39 different mutations in the *TrkA* gene had been identified in patients from different countries by 2006 [9, 15]. These mutations include frameshift, nonsense, splice, and missense mutations involving the extracellular NGF-binding domain or the intracellular signal-transducing domain [9, 15, 71, 82]. Mardy's group has shown that some of the previously identified mutations result in decreased autophosphorylation of the TrkA receptor [84]. Though the mutations are localized either in the regions coding for the extracellular binding of NGF or the intracellular signal-transducing domains of TrkA, no differences in phenotype were observed [22, 64, 71, 80]. Anand suggests that these mutations result in partial loss-of-function, since axon-reflex vasodilation and sweating can be elicited in HSAN4 patients (though the responses are reduced) [72]. It is likely that these mutations decrease or prevent the formation of correctly configured high-affinity binding sites for NGF, thus leading to decreased receptor activation [85].

Thus, defects in NGF signal transduction caused by receptor mutations in HSAN4 patients leads to loss of NGF-dependent neurons, probably in great part due to apoptosis during development [22].

HSAN5: TrkA and NGFB Mutations

Houlden et al. reported on a consanguineous Pakistani family with HSAN5 in 2001. They sequenced the *TrkA* gene and found an exon 8 mutation of Tyr359Cys [8, 86, 87]. It has been suggested that the group of HSAN5 patients with these mutations may have an allelic variation of HSAN4 [4, 9, 15, 88].

Subsequently, however, Toscano et al. sequenced the *TrkA* gene of a girl with clinically and neurophysiologically-diagnosed HSAN5 [89]. They did not find a mutation, indicating that another gene was likely responsible for her disease. A different group performed a linkage study of a consanguineous Swedish family with a HSAN5 phenotype, which showed an autosomal recessive inheritance pattern and demonstrated a locus in an 8.3-cM region on chromosome 1p11.2-p13.2 [26]. Candidate gene analysis showed a mutation in the coding region of the *NGFB* gene, which was specific for the disease haplotype [9, 26]. They found a point mutation (661 C→T) in exon 3 of a conserved region of the *NGFB* gene in three severely affected cases of HSAN5 [9, 25, 26]. These family members were homozygous for this haplotype. The same disease haplotype was also present in some mildly affected and unaffected family members, but these individuals were only heterozygotes. This mutation resulted in a substitution of arginine to tryptophan at position 211 in the NGFB polypeptide, which corresponds to amino acid 100 of the mature protein. This amino acid is located in a region that is highly conserved among the other human neurotrophins (brain derived neurotrophic factor, neurotrophin 3 and neurotrophin 4/5) and also in the NGF molecules of different species. The fact that these molecules all bind the p75 receptor (the low-affinity NGF receptor) suggests that this region is important for binding to and/or activation of this receptor [9, 26]. This group proposed that the mutation diminished (but did not eliminate) the ability of NGF to activate the TrkA receptor. An alternative hypothesis was derived from laboratory experience with knockout mice for the p75 receptor. These mice have a milder phenotype than knockouts

for TrkA or NGF. Thus, it is possible that the NGF mutation identified in these patients affects the ability to activate the low-affinity receptor, leading to milder disease than HSAN4 [25, 26].

HSAN 2: Locus Unidentified at the Onset of Our Study

The genes for HSANs 2 and 5 had not yet been identified at the start of this study. This thesis details the first steps in our quest to map and clone the mutation responsible for Hereditary Sensory and Autonomic Neuropathy Type 2.

Our knowledge of the mutated genes for HSANs 1, 3 and 4 at the onset of the study suggested that broad categories of genes that could be mutated in HSAN2. It also stressed that numerous molecules and cellular interactions are at play to ensure proper neuronal development and survival. Mutations in the *SPTLC1* gene (HSAN1) clearly suggest that neuronal metabolism of sphingolipids is important, while mutations in the *TrkA* gene (HSAN4) point to the importance of neurotrophic factors in sensory neuronal survival. Lastly, though less conclusively, mutations in *IKBKAP* (HSAN3) suggest that neuronal-specific transcription factors may play key roles in neuronal development and survival. Together, these findings underlined the great number of genes that could be candidate for mutations in HSAN2. This consequently limited the use of a candidate gene-screening strategy as opposed to a traditional positional cloning approach in order to uncover the causal mutation for HSAN2.

The discovery of a large cohort of French-Canadian cases of HSAN2 opened the way to launching this positional cloning strategy. The first cases of HSAN2 were identified through their treating physicians in the Lanaudière region of Québec. These individuals and others from southern Québec were recruited for the study with the objective of mapping the responsible locus and eventually identifying the mutated gene.

The first chapter of this thesis reviews the previously published clinical literature on Hereditary Sensory and Autonomic Neuropathy Type 2, and details the cases with their epidemiologic, clinical and laboratory features. The second chapter, an article published by

our group in the journal *Neurology* in 2005, describes the clinical features and geographic distribution of these French-Canadian cases, demonstrates that HSAN2 is not linked to any of the three loci of HSAN 1, 3 and 4 and describes the specific *HSN2* mutations identified in this cohort [90].

Table I

Hereditary Sensory and Autonomic Neuropathies: Clinical Features [5, 91]

HSAN Type	1	2	3	4	5
Mode of Inheritance	Autosomal dominant	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive
Age of Onset	2nd to 4th decades	Infancy?	Infancy	Infancy	Infancy
Temperature Sensation	↓	↓	↓	↓	↓
Pinprick Sensation	Distal ↓	Distal ↓	↓	-	-
Tactile Sensation	N to ↓	Distal ↓	↓	N	N
Position Sense	N to ↓	↓	N to ↓	N	N
Facial Hypoesthesia	-	-	-	+	-
Trunk Hypoesthesia	-	-	+	-	-
Symmetry	+	+	-	-	-
Corneal Reflex	?	↓	↓	↓	?
Muscle Strength	↓	N	N	N	N
Deep Tendon Reflexes	N to ↓	↓	↓	N	N
Postural Hypotension	-	-	+, episodic	-	-
Episodic Fevers	?	±	+	+	±
Alacrima	-	-	+	-	-
Sweating	N to ↓	N	↑	↓ to -	N
Gastrointestinal Dysmotility	?	+	+	-	?
Intelligence	N to ↓	N	N	↓	N
Progression	Slow	?	?	?	?

N = normal; ↑ = increased; ↓ = decreased; ↓↓ = markedly decreased; ± = variable; – = absent; + = present; ? = uncertain/unknown

Other Features:	
Type 1	Genetic heterogeneity: autosomal recessive and X- linked transmission also reported
Type 2	More generalized sensory deficit to all modalities, but face and trunk not or little affected
Type 3	History of poor feeding, vomiting and failure to thrive; esophageal dysmotility; unexplained fevers; skin blotching; kyphosis/scoliosis; ataxia; diminished visual acuity; Ashkenazi Jewish descent
Type 4	Episodic high fevers in infancy; skin blotching; mutilation of oral structures e.g. tongue

Table II

Hereditary Sensory and Autonomic Neuropathies: Electromyographic and Pathologic Features [5, 91]

HSAN Type	1	2	3	4	5
EMG Sensory Nerve Conduction Velocity	low N to ↓	low N to slightly ↓	-	N	-
EMG SNAP	↓	-	-	N	N
EMG Motor Nerve Conduction Velocity	low N to ↓	low N to slightly ↓	↓	N	N
Biopsy:					
Myelinated Fibers	↓ small fibers; ↓↓ large fibers	↓↓ to -	↓↓	↓	↓↓ small fibers
Unmyelinated Fibers	↓ to ↓↓	↓	↓↓	↓↓ to -	±

N = normal; ↓ = decreased; ↓↓ = markedly decreased; ± = variable; - = absent; SNAP = sensory nerve action potential

Table III

Hereditary Sensory and Autonomic Neuropathies: Identified Loci and Genes [5, 12, 91]

HSAN Type	Locus	Year Mapped	Mutated Gene	Year Identified	Gene product
1	9q22.1-q22.3	1996	*SPTLC1*	2001	Enzyme
2	12p13.33	2004	*HSN2*	2004	Novel protein
3	9q31-q33	1993	*IKBKAP*	2001	Transcription factor
4	1q21-q22	1990	*TrkA*	1990	Neurotrophin receptor
5	1q21-q22	2001	*TrkA*	2001	Neurotrophin receptor
	1p11.2-p13.2	2004	*NGFB*	2004	Neurotrophin

Pedigree of Family D of Heller and Robb [35]

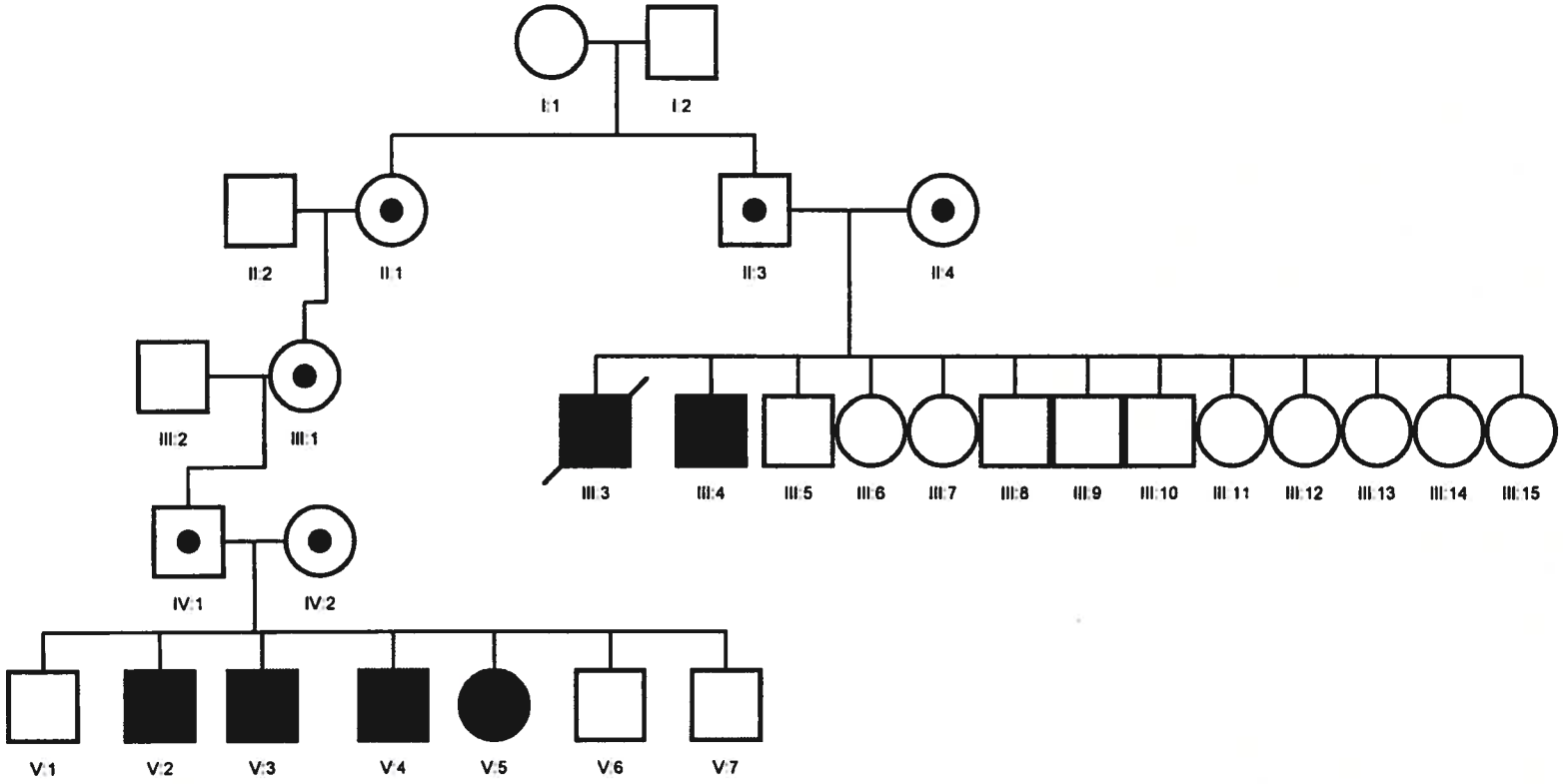

Figure 1. Pedigree of Family D of Heller and Robb, 1955. Shaded forms represent affected family members. Dots represent probable carriers of the disease.

FIRST CHAPTER: REVIEW OF THE LITERATURE ON HSAN2

CLINICAL PHENOTYPE OF HSAN2

This chapter consists of a review of the existing literature on the HSAN2 phenotype at the onset of our study. Our objectives were to identify all likely cases of HSAN2 described in the literature and to better characterize its phenotype, in order to ensure that our entire study population was clinically homogeneous. References were found through Medline searches and in bibliographies of the principal references on this disease, which has also been known as neurogenic acroosteolysis, Giaccai type acroosteolysis, hereditary sensory radicular neuropathy (recessive form), progressive sensory neuropathy of children, and congenital sensory neuropathy (MIM 201300). Particularly useful were the landmark article by Ohta and Dyck, in which the entity was clearly defined for the first time, as well as the review by Dyck of the HSANs in the textbook *Peripheral Neuropathy* [2, 5]. The historical and clinical features were evaluated in each case and compared to the characteristic features established by Ohta and Dyck. We then divided the cases into two main groups based on these features:

1) Definite cases of HSAN2 (Table IV, p. 30)
2) Cases clearly misclassified as HSAN2 (Table V, p. 31)

Epidemiology of HSAN2

The epidemiologic characteristics of published definite HSAN2 cases are summarized in table VI (p. 32). With regards to ethnic origin, five of the families were French-Canadian, two were French, one was from Newfoundland, one was from Nova Scotia, and two were American. Overall, twelve of 24 patients (50%) were of French-Canadian origin. In keeping with the known pattern of autosomal recessive transmission, a history of parental consanguinity was present in two families. Males and females were both affected, although there seems to be

a male preponderance among these published cases (fifteen males to nine females = 63% males). Furthermore, the average percentage of affected siblings in the literature is 48%, which is higher than the expected 25%. This could be explained in part by the fact that some deceased or non-affected individuals may not have been reported.

Homogeneity of Clinical Phenotype of HSAN2

An analysis of the clinical phenotype of the typical HSAN2 cases reveals a striking homogeneity of phenotype (Table VII, p. 33-35). Age of onset (defined as the age at first soft tissue infection or fracture, or less frequently as the time sensory deficit was first recognized either by the patient, parents or a physician), was almost uniformly during childhood, usually in preadolescence (mean = 7 years; range = 2-13). Deep tendon reflexes were decreased or absent in all cases. On sensory examination, all modalities were affected in the extremities, with little or no deficit in facial or trunk sensation. Progression of disease was not mentioned in most articles, but Bonnett's 1921 article details the follow-up of a patient whose case was first published in 1909 [92, 93]. He noted that on the initial exam, there was anesthesia to tactile and to pinprick sensation in the foot and inferior third of the leg; hypoesthesia continued up to the knee, but sensation above the knee was strictly normal [92]. However, the exam in 1920 showed complete anesthesia to pinprick sensation to four finger widths *above* the knee [93]. Murray noted that one of the sisters discussed in his publication had been examined at a 26-year interval, without demonstrable progression of sensory deficit [34]. Ohta mentioned that the disease was either static or had a slow progression [2]. On the other hand, the extent of mutilation of the extremities seemed to follow a clearly progressive course in most patients [92]. Autonomic manifestations were not predominant and patients had no motor disturbances or cognitive deficits.

PATHOLOGIC PHENOTYPE OF HSAN2 (Table VIII, p. 36)

The literature regarding the pathologic findings in HSAN2 is not extensive, and consists of isolated peripheral nerve biopsies in a few patients. Parks' 1945 article noted only the absence of inflammatory changes in a small nerve of the foot [31]. Heller noted marked demyelination around axis cylinders, without evidence of interstitial hypertrophy or cellular proliferation in the right lateral femoral cutaneous nerve of one of his subjects, which is suggestive of a purely degenerative process [35]. Dyck mentioned that the left sural nerve of one of his patients was small, and contained only a single myelinated fiber. However, he also noted that there was no evidence of demyelination or remyelination, and a lack of endoneurial proliferation. This nerve also contained an unusually large number of small unmyelinated fibers [30]. Murray examined the femoral cutaneous nerve of one patient and found only one myelinated fiber in sections, and again, no evidence of degeneration or of reactive change. He also mentioned groups of axons surrounded by single Schwann cells [34].

Ohta and Dyck's 1973 article clearly provides the best and most thorough pathologic description of HSAN2 to date [2]. In the sural nerves of subjects one to four, they noted diminished transverse fascicular areas, along with almost complete loss of myelinated fibers. Again, no evidence of myelin breakdown products or site of former myelinated fibers was found to suggest an active degenerative process. There was an increased amount of collagen, and vacuolated fibroblasts were seen in the endoneurium. In addition, unmyelinated fibers were morphologically abnormal in size, arrangement and distribution. The axoplasm was abnormal, the most frequent anomalies being the presence of densely packed tubular structures, dense bodies, cored vesicles and vacuoles in axonal processes. Furthermore, in the lateral fascicle of the deep peroneal nerve of one of his subjects, 13 of the 200 myelinated fibers found showed some evidence of demyelination and remyelination, which suggests a possible active process of fiber degeneration. On the other hand, endoneurial capillaries, as well as epineurium and

perineurium were normal. The authors also noted normal internode lengths in the fibers studied [2].

Studies of the other types of HSAN suggest a pathologic process that also affects neuron cell bodies in the dorsal root ganglia (DRG). It has been shown in HSAN3, for example, that fetal development and post-natal maintenance of DRG neurons is abnormal, with significantly diminished numbers and marked reduction in size of DRGs. Continued depletion of unmyelinated sensory and autonomic neurons with age has also been shown [17, 18]. Superior cervical ganglia are also smaller, with a diminished number of neurons [17]. Additionally, in the mouse model of HSAN4, affected animals have loss of almost all DRG neurons associated with nociceptive functions. They have extensive neuronal loss in sympathetic ganglia as well [61]. These findings may eventually be confirmed in HSAN2 patients or in an animal model of HSAN2; however, data on this subject remains anecdotal. One of the cases described by Heller in 1955 underwent a DRG biopsy, analyzed by Dr. Masson of the Hôtel-Dieu de Montréal hospital [35]. A severe decrease in the number of cells in the DRG was noted (Heller, personal communication, 2000).

ELECTROPHYSIOLOGIC FINDINGS IN HSAN2 (Table IX, p. 37)

The published electrophysiologic findings in HSAN2 cases also show a typical profile. Motor conduction is always in the low normal to normal range. Sensory nerve action potentials are uniformly absent, and conduction velocity is not measurable [2, 30, 34]. The only exception is one of Hould's cases, a twelve-year old who had a normal sensory conduction velocity [1].

PUBLISHED CASES CLEARLY MISCLASSIFIED AS HSAN2 (Table V, p. 31)

There were many cases found in our review of the literature that were identified as possible cases of HSAN2; however, upon closer inspection with the criteria established above, these individuals exhibited many traits divergent from the typical HSAN2 clinical phenotype. These patients were clearly not affected by the same disorder. The cases, as well as the criteria for exclusion, are summarized in Table V (p. 31).

DISCREPANCIES BETWEEN THE CLASSIFICATION OF DYCK AND THE PRESENT CLASSIFICATION (Tables V and X, pp. 31, 38)

In our classification, published cases were strictly evaluated according to the criteria suggested by Peter Dyck (enumerated in the introduction to this paper)[5]. These criteria were further confirmed in the cases evaluated in our Québec cohort who also lacked any significant neurological or congenital defects (see Chapter 2). Thus, any significant additional neurologic deficit or congenital systemic disease was considered to be a criterion for exclusion. This was done in order to ensure a homogeneous population for the subsequent linkage analysis. It is entirely possible that these other cases are variants of HSAN2, associated with different mutations.

Definite Cases of HSAN2 (Table IV, p. 30)

The cases published in 1973 by Murray were considered to be possible cases by Dyck, but were classified by our group as definite cases of HSAN2. These were two sisters who presented the same clinical picture; however, the first had trunk hypoesthesia in addition to the limb sensory deficit, a feature that was not present in the other more typical cases of HSAN2, or in our Québec cohort[34]. Nevertheless, this patient had confirmatory EMG findings with normal

motor conduction and no demonstrable sensory nerve action potentials. Lateral femoral cutaneous nerve biopsy showed only one myelinated nerve fiber. There was "no evidence of degenerative or reactive change" [34]. This leads us to conclude that these women in fact very likely suffered from HSAN2.

Cases Probably Misclassified as HSAN2 (Tables V and X, pp. 31, 38)

The case of Head was classified as a possible case in our study, despite Dyck's classification as HSAN2 because of the predominant ataxia, which is not at all typical of other HSAN2 cases [94].

The two cases published by Adams were classified as possible cases of HSAN2 by Dyck, but were excluded by our criteria. The article described two siblings, the first of whom exhibited complete sensory loss to all modalities in the extremities as well as in the face and trunk. Deep tendon reflexes were absent. The first patient also had anhidrosis, postural hypotension, and a history of temperature rise with exercise. He also had an ataxic gait. His sister had died at twenty-nine years of age following a fracture and osteomyelitis; she also had severe sensory loss and ataxic gait [95]. These two siblings clearly exhibited features atypical for the disease as described to date. We therefore believe that they presented a distinct disorder.

Bousquet's case was classified as possible HSAN2 by Dyck, but excluded by us because of the normal tactile sensation, which is also atypical of the disease [96].

Cruchet's case was also classified by Dyck as a possible case of HSAN2. Despite sharing many clinical features of HSAN2, it was excluded in our classification because of atrophy and weakness of extremities and a low IQ [97].

Dyck's 1983 case is doubtful because of the presence of sphincter disturbance, alacrima, facial and trunk hypoesthesia, as well as ataxia [3].

Johnson's two patients were also excluded in our HSAN2 classification because the first patient was deaf, and the second patient had anhidrosis, trunk hypoesthesia, and a low IQ [98].

Miller's two patients, who were classified as HSAN2 by Dyck, had tonic pupils and vibration and position sense were more affected than pinprick and temperature. They also had muscle atrophy, with upper extremities being more affected than lower extremities. The first patient was deaf. These characteristics separated them from the typical cases [99].

Parks' second patient was excluded in our classification despite being classified as HSAN2 by Dyck because of lower extremity muscle weakness. Her deep tendon reflexes were also merely diminished, whereas most HSAN2 patients have abolished reflexes after childhood [31].

Pinsky's three cases were excluded in our classification due to several criteria, including low IQ, paroxysmal fevers in the first patient, low IQ and normal myelination on nerve biopsy in the second patient, and retarded early development, autistic behavior, and dysphonia in the third patient [100].

Schoene's two patients were also considered to be unlikely cases of HSAN2 in our classification based on presence of lightning pains in the first patient, absent corneal reflexes and joint laxity in the second patient, and merely decreased (rather than absent) deep tendon reflexes in both patients [101].

Wadia's patient had decreased facial and trunk sensation as well as decreased muscle strength and atrophy. He also had unilateral preservation of the Achilles' reflex. These traits are clearly not part of the established HSAN2 criteria, and these individuals were therefore excluded as definite cases [102].

Finally, Winkelmann's patient, which was also classified as an HSAN2 case by Dyck, was excluded in our classification because of deafness, decreased facial and trunk sensation, diminished corneal reflex and ataxia. He also had tongue deviation and atrophy [103].

CONCLUSION

In summary, the diagnostic criteria for HSAN2 have been well established in the literature, most notably by Dyck and co-workers. The published data also indicates that this autosomal recessive disease seems to occur predominantly (50 % of published cases) among individuals of French-Canadian descent.

The next chapter details the identification of a Québec cohort of HSAN2 cases who uniformly meet the aforementioned criteria. It will also be shown that HSAN2 is not linked to the chromosomal loci of HSANs 1, 3 and 4. Finally, two distinct mutations are identified as being present in the French-Canadian cases of the disease.

Table IV

Definite Published Cases of HSAN2

Author	Year of Publication	Number of Cases	Comments
Bonnett [92]	1909	1	
Price [104]	1913	3	
Bonnett [93]	1921	1	*Same patient as Bonnett 1921
Barbier [105]	1931	1	*Same patient as Bonnet 1909 and 1921
Parks [31]	1945	1	
Delmas- Marsalet [106]	1946	1	
Ogryzlo [32]	1946	4	
Lessard [33]	1953	2	
Heller [35]	1955	6	
Dyck [30]	1966	4	
Hould [1]	1967	4	*Same patients as Dyck 1966
Murray [34]	1973	2	
Ohta [2]	1973	3	*Same cases as patients 2, 3 and 4 of Dyck 1966

Total = 24 patients; French-Canadian = 12 (50 %)

Table V

Published Cases Clearly Misclassified as HSAN2

Author	Year of Publication	Number of Cases	Exclusion Criteria
Morvan [6]	1883	1	Paresis, muscle atrophy, asymmetrical deficit: probable syringomyelia
Cruchet [97]	1920	1	Low IQ
			Atrophy and weakness of extremities
McMurray [107]	1950	1	Normal deep tendon reflexes
			Face hypoesthesia; absent corneal reflexes; weak gag reflex
			* Same patient as Baxter 1960
Gaté [108]	1936	1	Normal deep tendon reflexes
Thevenard [109]	1942		HSAN1 according to Dyck, dominant transmission, normal to increased deep tendon reflexes
			Upper limbs almost normal in second patient
Andre [110]	1949	5	Probable syringomyelobulbia in first two patients and syringomyelia in other three
Denny-Brown [111]	1951	1	Probable HSAN1: lower limbs more affected than upper
			Deafness, lightning pains, sphincter disturbance, prominent ataxia
Silverman [112]	1959	2	Facial hypoesthesia to pinprick in first patient
			Deep tendon reflexes normal, weak corneal reflex; joint laxity in second patient
Baxter [113]	1960	1	Probable Friedrich's ataxia: deafness, normal deep tendon reflexes
Johnson [98]	1964	2	Deafness in first patient
			Low IQ in second patient
			Trunk hypoesthesia
Swanson [114]	1965	2	HSAN4 in first patient according to Dyck
			Tactile sensation normal in both patients
Haddow [115]	1970	2	Facial and trunk hypoesthesia
			Low IQ in first patient
			Severe dysgraphia in second patient
Adams [95]	1973	2	Postural hypotension, hyperthermia upon exercise, trunk and face hypoesthesia, sensory ataxia
Jedrejowska [116]	1976	2	Lower limb hyperreflexia
			Upper limbs not affected
Nukada [117]	1982	4	Lightning pains in first and fourth patients, sphincter disturbance in third and fourth patients
			Anhidrosis, absent extraocular muscle function, merely diminished deep tendon reflexes in fourth patient
			Muscle weakness in second, third and fourth patients
			Normal deep tendon reflexes, except for Achilles' in second patient

Table VI
Epidemiologic Characteristics and Classification of Published Cases of HSAN2

First Author	Year	Case No.	Family	Gender	Parental Consanguinity	Number of Affected Siblings of Total	Transmission	Origin	Dyck 1993 HSAN2 (+/-)	Thomas 2007 HSAN2 (+/-)
Bonnett [92]	1909	1	A	F	N/A	1 of 3	?	French	-	+
Price [104]	1913	1	B	M	-	3 of 5	?	Amer.	-	+
Price	1913	2	B	F	-	3 of 5	?	Amer.	-	+
Price	1913	3	B	M	-	3 of 5	?	Amer.	-	+
Barbier [105]	1931	1	A	F	N/A	1 of 3	?	French	-	+
Parks [31]	1945	1	C	M	-	1 of 2	?	Amer.	+	+
Delmas- Marsalet [106]	1946	1	D	M	-	1 of 1	?	French	-	+
Ogryzlo [32]	1946	1	E	M	-	4 of 12	AR	Nfld.	+	+
Ogryzlo	1946	2	E	M	-	4 of 12	AR	Nfld.	+	+
Ogryzlo	1946	3	E	F	-	4 of 12	AR	Nfld.	+	+
Ogryzlo	1946	4	E	M	-	4 of 12	AR	Nfld.	+	+
Lessard [33]	1953	1	F	F	+*	2 of 10	AR	FrCan	+	+
Lessard	1953	2	F	F	+*	2 of 10	AR	FrCan	+	+
Heller [35]	1955	1	G	M	-	4 of 7	AR	FrCan	+	+
Heller	1955	2	G	F	-	4 of 7	AR	FrCan	+	+
Heller	1955	3	G	M	-	4 of 7	AR	FrCan	+	+
Heller	1955	4	G	M	-	4 of 7	AR	FrCan	+	+
Heller	1955	5	H	M	-	2 of 13	AR	FrCan	+	+
Heller	1955	6	H	M	-	2 of 13	AR	FrCan	+	+
Dyck [30]	1966	1	I	M	-	4 of 6	AR	FrCan	+	+
Dyck	1966	2	I	M	-	4 of 6	AR	FrCan	+	+
Dyck	1966	3	I	F	-	4 of 6	AR	FrCan	+	+
Dyck	1966	4	I	M	-	4 of 6	AR	FrCan	+	+
Hould [1]	1967	1	I	M	-	4 of 6	AR	FrCan	+	+
Hould	1967	2	I	M	-	4 of 6	AR	FrCan	+	+
Hould	1967	3	I	F	-	4 of 6	AR	FrCan	+	+
Hould	1967	4	I	M	-	4 of 6	AR	FrCan	+	+
Murray [34]	1973	1	J	F	+*	N/A	AR	NS	POSS	+
Murray	1973	2	J	F	+*	N/A	AR	NS	POSS	+
Ohta [2]	1973	1	I	M	-	4 of 6	AR	FrCan	+	+
Ohta	1973	2	I	F	-	4 of 6	AR	FrCan	+	+
Ohta	1973	3	I	M	-	4 of 6	AR	FrCan	+	+

M = male; F = female; + = yes; - = no; POSS = possible; N/A = not available; R = recessive; AR = Autosomal recessive
Amer.= American; Nfld.= Newfoundland; NS= Nova Scotia; FrCan= French-Canadian; *= parents first degree cousins

Table VII
Clinical Characteristics of Published Cases of HSAN2

First Author	Year	Case No.	Age of Onset	Facial Sensation	Muscle Strength	Biceps Reflex	Triceps Reflex	Patellar Reflex	Achilles' Reflex
Bonnett [92]	1909	1	13	?	N	-	-	-	-
Price [104]	1913	1	13	?	?	?	?	?	?
Price	1913	2	8	N	N	-	-	-	-
Price	1913	3	?	?	?	?	?	?	?
Barbier [105]	1931	1	12	?	N	-	-	-	+?
Parks [31]	1945	1	6	↓	?	-	-	-	-
Delmas-Marsalet [106]	1946	1	7	?	N	↓	↓	↓	-
Ogryzlo [32]	1946	1	Childhood	N	N	-	↓	-	-
Ogryzlo	1946	2	8	N	N	-	↓	-	-
Ogryzlo	1946	3	7	N	N	↓	N	-	-
Ogryzlo	1946	4	?	?	?	?	?	?	?
Lessard [33]	1953	1	3	?	?	-	-	↓	?
Lessard	1953	2	5	?	?	N	N	N	N
Heller [35]	1955	1	9	N	N	-	-	-	-
Heller	1955	2	6	N	N	-	↓	-	-
Heller	1955	3	7	N	N	-	-	-	-
Heller	1955	4	12	N	N	↓	N	N	N
Heller	1955	5	Childhood	N	N	↓	↓	-	-
Heller	1955	6	?	?	?	?	?	?	?
Dyck [30]	1966	1	2	N	N	↓	↓	-	-
Dyck	1966	2	Under 10	N	N	-	-	-	-
Dyck	1966	3	9	N	N	-	-	-	-
Dyck	1966	4	3	?	?	-	-	-	-
Hould [1]	1967	1	2	N	?	-	-	-	-
Hould	1967	2	9	N	?	-	-	-	-
Hould	1967	3	8	N	?	-	-	-	-
Hould	1967	4	3	?	?	?	?	?	?
Murray [34]	1973	1	2	N	N	-	-	-	-
Murray	1973	2	Childhood	N	N	-	-	-	-
Ohta [2]	1973	1	11	↓	N	↓	↓	-	-
Ohta	1973	2	9	N	N	↓	↓	-	-
Ohta	1973	3	6	N	N	↓	↓	-	-

N = normal; + = yes; - = no or absent; ↓ = diminished; ? = not mentioned/unknown; POSS=possible

Table VII (continued)

Clinical Characteristics of Published Cases of HSAN2

First Author	Year	Case No.	Tactile Sensation	Pinprick Sensation	Vibration Sense	Position Sense	Facial Hypoesthesia	Trunk Hypoesthesia	Progression of Sensory Deficit
Bonnett	1909	1	↓	↓	?	?	?	-	+
Price	1913	1	?	?	?	?	?	?	?
Price	1913	2	↓	↓	↓	↓	-	-	?
Price	1913	3	?	?	?	?	?	?	?
Barbier	1931	1	↓	↓	?	?	?	-	?
Parks	1945	1	↓	↓	↓	↓	+	-	?
Delmas- Marsalet	1946	1	?	↓	?	?	-	-	?
Ogryzlo	1946	1	↓	↓	↓	↓	+	-	?
Ogryzlo	1946	2	↓	↓	↓	↓	-	-	?
Ogryzlo	1946	3	↓	↓	N	↓	-	-	?
Ogryzlo	1946	4	?	?	?	?	?	?	?
Lessard	1953	1	↓	↓	?	?	?	?	?
Lessard	1953	2	?	?	?	?	?	?	?
Heller	1955	1	↓	↓	↓	↓	-	-	?
Heller	1955	2	↓	↓	↓	↓	-	-	?
Heller	1955	3	↓	↓	↓	↓	-	-	?
Heller	1955	4	↓	↓	N	N	-	-	?
Heller	1955	5	↓	↓	?	↓	-	+	?
Heller	1955	6	?	?	?	?	?	?	?
Dyck	1966	1	↓	↓	↓	↓	-	-	?
Dyck	1966	2	↓	↓	↓	↓	-	-	?
Dyck	1966	3	↓	↓	↓	↓	-	-	?
Dyck	1966	4	↓	↓	↓	↓	?	-	?
Hould	1967	1	↓	↓	?	?	-	-	?
Hould	1967	2	↓	↓	?	↓	-	-	?
Hould	1967	3	↓	↓	?	↓	-	-	?
Hould	1967	4	?	?	?	?	?	?	?
Murray	1973	1	↓	↓	↓	↓	-	+	-
Murray	1973	2	↓	↓	↓	↓	-	-	-
Ohta	1973	1	↓	↓	↓	↓	+	+	POSS
Ohta	1973	2	↓	↓	↓	↓	-	-	POSS
Ohta	1973	3	↓	↓	↓	↓	-	-	POSS

N = normal; + = yes; - = no or absent; ↓ = diminished; - = absent; ? = not mentioned/unknown; POSS=possible

Table VII (continued)
Clinical Characteristics of Published Cases of HSAN2

First Author	Year	Case No.	Comments:
Bonnett	1909	1	
Price	1913	1	Symptoms almost identical to patient 2
Price	1913	2	
Price	1913	3	Symptoms almost identical to patient 2
Barbier	1931	1	Same patient as Bonnett 1909 and 1921
Parks	1945	1	Slight facial hypoesthesia
Delmas- Marsalet	1946	1	Upper limbs normal; presence of lightning pains; slight muscular atrophy
Ogryzlo	1946	1	Diminished pinprick in lower limbs and face
Ogryzlo	1946	2	
Ogryzlo	1946	3	
Ogryzlo	1946	4	Deceased at 37 years (six years before publication)
Lessard	1953	1	Loss of all toes before age thirteen years
Lessard	1953	2	Upper limbs morphologically normal; sensory exam: probable hypoesthesia
Heller	1955	1	Dominant transmission; tactile and pinprick sensation more affected than position and vibration
Heller	1955	2	Vitiligo
Heller	1955	3	
Heller	1955	4	Tactile and pinprick sensation diminished over hands and feet
Heller	1955	5	Spontaneous loss of distal half of right foot
Heller	1955	6	Deceased at fifteen after amputation of infected toe; same complaints as brother (case number 5)
Dyck	1966	1	Left sural nerve bx: no identifiable myelinated axons
Dyck	1966	2	
Dyck	1966	3	
Dyck	1966	4	Gradient for hypoesthesia, but marked impairment
Hould	1967	1	
Hould	1967	2	
Hould	1967	3	
Hould	1967	4	
Murray	1973	1	
Murray	1973	2	One sister examined at 26-year interval without progression
Ohta	1973	1	
Ohta	1973	2	
Ohta	1973	3	Milder hypoesthesia

Table VIII

Pathologic Phenotype of Published Cases of HSAN2

Author	Year of Publication	Case No.	Nerve biopsied: Pathology
Bonnett [92]	1909	1	-
Price [104]	1913	1	-
Barbier [105]	1931	1	-
Parks [31]	1945	1	-
Delmas-Marsalet [106]	1946	1	-
Ogryzlo [32]	1946		-
Lessard [33]	1953		-
Heller [35]	1955		Right lateral femoral cutaneous nerve: Marked demyelination around axis cylinders No interstitial hypertrophy nor cellular proliferation
Dyck [30]	1966	1	Left sural nerve: Small nerve trunk, one identifiable small myelinated fiber No evidence of myelin breakdown or remyelination, no proliferation of endoneurium Large number of small umyelinated fibers
Hould [1]	1967		Same as Dyck 1966
Murray [34]	1973	1	Right lateral femoral cutaneous nerve: One myelinated fiber in sections No evidence of degeneration or reactive change, no proliferation of endoneurium Groups of axons surrounded by single Schwann cells
Ohta [2]	1973	1,2,3,4	Sural nerves: Diminished transverse fascicular areas Almost complete loss of myelinated fibers Increased collagen No myelin breakdown products, nor evidence of site of former myelinated fibers Vacuolated fibroblasts in endoneurium Normal epineurium, perineurium and endoneurial capillaries Aggregates of Schwann cells and proceses Excessive irregularity of Schwann cell cytoplasm Abnormal arrangement, size and distribution of unmyelinated fibers Abnormal axoplasm: presence of densely packed tubular structures, dense bodies, cored vesicles and vacuoles in axonal processes
Ohta	1973	2	Deep peroneal nerve: Some demyelination and remyelination (13 of 200 myelinated fibers studied) Normal internode lengths

Table IX

Electrophysiologic Phenotype of HSAN2

Author	Year of Publication	Patient No.	Motor Conduction Velocity	Sensory Conduction Velocity	SNAP
Bonnett [92]	1909	1	N/A	N/A	N/A
Price [104]	1913	1	N/A	N/A	N/A
Barbier [105]	1931	1	N/A	N/A	N/A
Parks [31]	1945	1	N/A	N/A	N/A
Delmas-Marsalet [106]	1946	1	N/A	N/A	N/A
Ogryzlo [32]	1946	-	N/A	N/A	N/A
Lessard [33]	1953	-	N/A	N/A	N/A
Heller [35]	1955	-	N/A	N/A	N/A
Dyck [30]	1966	1	N	-	-
Hould [1]	1967	1	N/A	N	N/A
Murray [34]	1973	1	N	-	-
Ohta [2]	1973	1	low N to N	-	-
		2	low N	-	-
		3	low N	-	-
		4	low N to N	-	-

N/A = not available; N = normal; - = absent; SNAP = sensory nerve action potential

Table X

Published Cases Probably Misclassified as HSAN2

Author	Year of Publication	Number of Cases	Exclusion Criteria
Bousquet [96]	1966	1	Normal tactile sensation
Dyck [3]	1983	1	Alacrima, facial and trunk hypoesthesia, ataxia
Head [94]	1903	1	Predominant ataxia
Miller [99]	1976	2	Tonic pupils, vibration and position sense more affected than pinprick and temperature
			Muscle atrophy, upper extremities more affected than lower
Parks [31]	1945	1	Muscle weakness in lower extremities, reflexes merely diminished
Pinsky [100]	1966	3	Low IQ, paroxysmal fever: probably Riley-Day in first patient
			Low IQ, normal myelination on nerve biopsy in second patient
			Retarded early development, autistic behavior, dysphonia in third patient
			Reflexes diminished to normal in second patient, merely diminished in other two
			Facial and trunk hypoesthesia in all three patients
			Tactile sensation normal in first patient, mentioned as "present" in second and third
			Alacrima in first and second patients
Schoene [101]	1970	2	Lightning pains in first patient
			Weak corneal reflex, joint laxity in second patient
			Deep tendon reflexes merely diminished in both patients
Wadia [102]	1960	1	Diminished facial and trunk sensation
			Decreased muscle strength, atrophy, Achilles' reflex present
Winkelmann [103]	1962	1	Deafness, ataxia
			Diminished facial sensation, diminished corneal reflex, trunk hypoesthesia
			Tongue deviation and atrophy

SECOND CHAPTER: TWO MUTATIONS IN THE *HSN2* GENE EXPLAIN THE HIGH PREVALENCE OF HSAN2 IN FRENCH CANADIANS

This chapter consists of the description of the cluster of HSAN2 patients discovered in Québec. It details the historical and clinical characteristics of the group, as well as the molecular studies performed on samples obtained from these patients. It is shown that that the HSAN2 locus is not localized at the same chromosomal loci as HSAN types 1, 3 and 4. Furthermore, three distinct mutations are identified in this cohort, which all present with an identical clinical phenotype within the group.

CO-AUTHOR CONTRIBUTIONS

Tina Thomas*: Recruitment of first fifteen patients, detailed evaluation and clinical description of these patients, hospital chart reviews, compilation of genealogies for first ten families, DNA isolation for first fifteen patients, linkage study, composition of article manuscript, conception of figures and tables.

Katel Roddier: Genotyping, haplotyping, linkage study to 12p13.33 locus, prediction of number of distinct mutations, identification and characterization of mutations, composition of article manuscript.

Geneviève Marleau: Analysis of results of two genome scans and fine mapping of positive regions.

Anne-Marie Gagnon: Production and analysis of data for second genome scan.

Marie-Josée Dicaire: Production and analysis of data for first genome scan (Weber 9 panel).

Anik St-Denis: Participation in analysis of results of two genome scans and in fine mapping of positive regions.

Isabelle Gosselin: Identification and characterization of the mutation in the case of Lebanese origin.

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* The aspects of the article which were exclusively the work of Ms. Roddier are discussed in her masters' thesis (Université de Montréal) and do not appear in the conclusion of this thesis. The contributions of the two first authors of this article are substantial and distinct (as stated in the published article), and are thus presented in both theses.

Anne-Marie Sarrazin: Clinical evaluation and reference of certain cases.

Albert Larbrisseau: Clinical evaluation and reference of certain cases.

Marie Lambert: Clinical evaluation and reference of certain cases.

Michel Vanasse: Clinical evaluation and reference of certain cases.

Daniel Gaudet: Clinical evaluation of certain cases. Helped in the study design as head of ECOGENE-21.

Guy Rouleau: Sharing of information on the *HSN2* locus before publication.

Bernard Brais: Recruitment of families, evaluation and clinical description of patients, supervision of genome scans, linkage studies and mutation studies, estimation of carrier frequencies, correction of manuscript.

TWO MUTATIONS IN THE HSN2 GENE EXPLAIN THE HIGH PREVALENCE OF HSAN2 IN FRENCH CANADIANS [90]

K. Roddier, BSc[a]*; T. Thomas, MD[a]*; G. Marleau, BSc[a]; A.M. Gagnon, BSc[a]; M.J. Dicaire, BSc[a]; A. St-Denis, MSc[a]; I. Gosselin, BSc[a]; A.M. Sarrazin, MD[b]; A. Larbrisseau, MD[c]; M. Lambert, MD[c]; M. Vanasse, MD[c]; D. Gaudet, MD PhD[d], G.A. Rouleau MD PhD[e], and B. Brais, MD PhD[a]

*These authors have contributed equally to this work

From the:

a. Laboratoire de neurogénétique, Centre de recherche du CHUM, Université de Montréal, Montréal, Québec, Canada
b. Centre Hospitalier Régional de Lanaudière, Saint-Charles-Borromée, Québec, Canada
c. Département de pédiatrie, Hôpital Sainte-Justine et Université de Montréal, Montréal, Québec, Canada
d. Centre de médecine génique communautaire de l'Université de Montréal, Centre Hospitalier de la Saguamie, Saguenay, Québec, Canada
e. McGill Health Centre, McGill University, Montréal, Québec, Canada

ABSTRACT

Background

Hereditary sensory and autonomic neuropathy type 2 (HSAN2, MIM 201300) is a rare recessive neuropathy usually diagnosed in the first decade. The 1973 study of a French-Canadian family led to the definition of HSAN2.

Objectives

To demonstrate that the apparent higher prevalence of HSAN2 in Québec is due to the presence of two *HSN2* mutations and that carriers of different mutations appear to have a similar phenotype.

Methods

Through attending physicians, the authors recruited French-Canadian patients with HSAN2. Exclusion of linkage to the known HSAN loci and linkage to the HSAN2 locus was performed using standard methods. Sequencing of the *HSN2* gene was used to uncover the causal mutations.

Results

A large cluster of HSAN2 patients comprising sixteen affected individuals belonging to thirteen families was identified. The mode of inheritance was clearly autosomal recessive. All patients originated from southern Québec, and 75% are from the Lanaudière region. Whereas linkage to the HSAN1, 3 and 4 loci was excluded, linkage to the 12p13.33 HSAN2 locus was confirmed. Sequencing of the *HSN2* gene uncovered two French-Canadian mutations and a novel nonsense mutation in a patient of Lebanese origin, all predicted to lead to truncations of the HSN2 protein. The comparison of clinical variables between patients with different genotypes did not suggest any difference in phenotype.

Conclusions

Two founder mutations are responsible for the apparent higher prevalence of HSAN2 in French Canadians. Genotype-phenotype correlation does not suggest any significant clinical variability.

INTRODUCTION

Hereditary sensory and autonomic neuropathies (HSANs) are a group of clinically and genetically heterogeneous disorders associated with sensory dysfunction. During the past decade, through positional cloning and candidate gene screening strategies, the loci and mutated genes have been identified for HSAN1 (MIM 162400), HSAN3 (MIM 223900), HSAN4 (MIM 256800) and HSAN5 (MIM 608654) [10, 26, 38, 47, 48, 49, 52, 78]. The mutated gene for HSAN2 (neurogenic acro-osteolysis, MIM 201300) has only recently been identified [118]. HSAN2 is a rare recessive disease that was first clearly described in 1973 in a French-Canadian kinship [1, 2]. In the literature, more than 50% of the patients described are of French-Canadian background [1, 2, 33, 25, 30]. The hallmarks of HSAN2 are an autosomal recessive mode of inheritance, onset of symptoms in infancy or early childhood, occurrence of distal extremity pathologies (paronychia, whitlows, ulcers and necrosis, Charcot joints), frequent amputations, sensory loss that affects all modalities of sensation (lower and upper limbs and perhaps the trunk as well), absence or diminution of tendon reflexes (usually in all limbs), minimal autonomic dysfunction, absence of sensory nerve action potentials (SNAP) and virtual absence of myelinated fibers with decreased numbers of unmyelinated fibers in sural nerves [2, 3]. Prior to this study, four loss-of-function mutations have been uncovered in the single-exon *HSN2* gene encoding a 434-amino acid open reading-frame [118, 119]. We have uncovered a large cohort of sixteen HSAN2 patients belonging to thirteen French-Canadian families. We have confirmed that all affected individuals have mutations in the *HSN2* gene. We describe the relative clinical homogeneity of this disease despite the differences in genotype.

METHODS

Patient Recruitment and Genealogies

Through attending physicians, we identified eighteen patients with HSAN2 belonging to thirteen families living in southern Québec. Sixteen subjects accepted to participate in this study following informed consent. Genealogic information was obtained at least up to grandparents in all families (except family M). Blood samples were collected for the sixteen subjects and their relatives. One Canadian patient of Lebanese origin was also recruited after informed consent.

Clinical Evaluation and Electromyography

All patients were evaluated with a standardized questionnaire and examination. Patient medical charts were reviewed. Previous electromyography (EMG) or nerve biopsy results done in different laboratories were compiled.

Genotype and Haplotype Analyses

DNA was extracted from blood samples using standard techniques [120]. Information about the seventeen polymorphic markers analyzed was obtained from the Genome Database (http://www.gdb.org/gdb). PCR was performed in 13 µL of a mixture containing 0.2 mM of dNTP-A, 0.025 mM of dATP, 0.16 µL of dATP ^{35}S labeled at 12.5 µCi/µL, 1X bovine serum albumin, Taq tampon 1X , 0.9 U of Taq polymerase, 1.15 µM of each primer, and 15 ng of DNA. The amplification procedure was performed with a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA), and it consisted of a hot-start of five minutes at 94°C followed by 35 cycles of denaturing at 94°C for thirty seconds, annealing at 52, 55 or 58°C for thirty seconds and elongation at 72°C for thirty seconds, and a final elongation at 72°C for five minutes; 2.5 µL was loaded on 5% polyacrylamide denaturing gels. Following electrophoresis,

the gels were dried and autoradiographs were obtained. The sizes of the different alleles were established by comparison with a standard M13 sequence prepared from the AmpliCycle Sequencing Kit (Applied Biosystems). Haplotypes were deduced from family data. The haplotyped carrier chromosomes were studied for all affected individuals, except for three individuals for whom no other familial samples were available (patients three, eight and the Lebanese patient). Some DNA samples were too limited to allow full genotyping (patient sixteen).

Sequencing

PCR primers were designed using PrimerSelect 4.03 (DNASTAR) and synthesized by Alpha DNA (Montréal, Canada). Fragments were amplified using the same amplification mix as for genotyping. Amplification was performed in a GeneAmp PCR System 9700 using the following PCR protocol: hot-start of five minutes at 94°C followed by 35 cycles of denaturing at 94°C for thirty seconds, annealing at 58°C for thirty seconds and elongation at 72°C for thirty seconds, and a final elongation at 72°C for five minutes. PCR products were sequenced at the Montréal Genome Center (Québec, Canada). Sequence traces were compared with the genomic sequence AB002342 from UCSC Genome Bioinformatics (http://genome.ucsc.edu) using SeqMan 4.03 (DNASTAR).

Mutation Carrier Frequencies

To estimate the regional mutation carrier frequencies for the two mutations, we based our calculation on the presence of five families with homozygotes for mutation *c.943C→T* and one for the rarer mutation *c.918-919insA* living in Lanaudière. We relied on the 1996 Canadian Census for the population of the county of Joliette. We excluded the population of the administrative region of the Municipalité Régional de Comté (MRC) des Moulins where no HSAN2 patients are known to live, because it consists largely of an urban suburb of Montréal

(population: 267 141 = 370 354 – 102 008). For the small town east of Joliette from which five families originate (one homozygote for mutation c.918-919insA, two homozygotes for mutation *c.943C→T* and two compound heterozygotes for mutations *c.943C→T* and *c.918-919insA*), the same Canadian 1996 census gives a population of 2 987 inhabitants. We applied Hardy-Weinberg proportions to estimate carrier frequencies because no regional population samples are available. Combined *HSN2* regional carrier frequencies for the two mutations were calculated by adding the individual frequencies for each mutation estimated by Hardy-Weinberg proportions.

RESULTS

French-Canadian HSAN2 Cluster

Our cohort comprises sixteen patients belonging to thirteen families (Fig. 2, p. 55). The most striking feature of this cohort is that all families come from the southern part of the province of Québec defined as southwest of an imaginary line along the Chaudière River that runs a few miles south of Québec City (Fig. 3A, p. 56). No patients have been diagnosed in Québec City or in the Saguenay-Lac-Saint-Jean region during the last twenty years (J. Mathieu and J.P. Bouchard, personal communications). Furthermore, 75% (twelve of sixteen) of patients originate from the Lanaudière region (Fig. 3A, p. 56). Joliette, the county town, is 75 kilometers (47 miles) north of Montréal. The southeastern part of the county along the Saint Lawrence River was first settled by French pioneers during the second half of the seventeenth century [121]. The majority of the population is still French-Canadian and lives in the agricultural southeastern part of the county. Unfortunately, the regional origin of the family described in 1973 is unknown and the family has been lost to follow-up (S. Verret and P.J. Dyck, personal communications). However, family I, originally described by Heller and Robb in 1955, also originated from the Lanaudière region [35]. Of the thirteen families, six have more than one

affected member (Fig. 2, p. 55). In family F, one daughter died of septicemia at age nineteen. In two families, parents are first-degree cousins; in one they are uncle and niece. Three families are known to be distantly related by more than one ancestor (Families A, E and F; Fig. 2). Five of the families originate from a small village east of Joliette. Last, Patient 8 lives northwest of Lanaudière, but his parents are first-degree cousins and their shared grandparents came from Lanaudière. To our knowledge, no parents are affected. Men and women are equally affected. Pedigree analysis strongly supports an autosomal recessive mode of inheritance (Fig. 2).

Identification of Two HSN2 Mutations in the French-Canadian Population

Linkage of the French-Canadian families with HSAN2 to the HSAN1, 3 and 4 gene loci was excluded by performing linkage analyses to polymorphic markers previously reported to be in close proximity to the three mutated genes (data not shown). A first genome scan using the Weber 8 panel of markers and a pooling strategy failed to uncover the disease locus. A second genome scan using a homozygosity mapping strategy also failed to identify the locus. Through collaboration with Xenon Genetics (Vancouver, Canada), we confirmed that our families were linked to the same 12q13.33 locus as two large consanguineous Newfoundland families (Table XI p. 56) [118]. Haplotype analysis determined that two distinct ancestral carrier chromosomes were present in our cohort. In retrospect, the presence of two mutations in our cohort and the absence of a close marker to the very telomeric *HSN2* gene in the Weber 8 marker panel, the telomeric D12S372 being more than 8 cM from the gene, together were responsible for the failure of the two genome scans performed under the assumption that one founder mutation was likely responsible for HSAN2 in most French-Canadian patients.

The *HSN2* gene was sequenced in all our sixteen patients, and two mutations were identified. Mutations detected in each individual were consistent with the haplotype analysis (Table XI p. 56). Mutation 1, *c.943C→T*, changes a CAG codon into a TAG stop codon and is predicted to truncate the protein to a 314-amino acid peptide. Mutation 2, *c.918-919insA*,

consists of the insertion of an A at position 918, causing a frameshift that leads to a premature truncation to a 318-amino acid peptide. Mutation 1 (*c.943C→T*) is the more frequent, being found on 75% (24/32) of mutation-carrying chromosomes. Fifty-six percent (9/16) of patients are *c.943C→T* homozygotes, 6% (1/16) are *c.918-919insA* homozygotes, and 38% (6/16) of patients are compound heterozygotes (*c.943C→T* and *c.918-919insA*) (Fig. 3B p. 55). Interestingly, the two families that have no known Lanaudière origin are homozygotes for the common *c.943C→T* mutation, suggesting that HSAN2 in patients outside the Lanaudière region will likely be caused by two copies of the common mutation. We also sequenced the DNA of a thirteen year-old Canadian child of Lebanese origin clinically affected with HSAN2 and identified that he was homozygote for a novel nonsense mutation: an 868C→T substitution at codon 290 of *HSN2*. This mutation is predicted to change a CGA coding for an arginine to a TGA stop codon that should lead to a 289-amino acid truncated protein.

Estimation of Carrier Frequencies in the Lanaudière Region

We are confident that we have identified all of the living HSAN2 patients in the Lanaudière region. Based on the 267 141 population of Lanaudière region, one can estimate the regional carrier frequencies to be in the order of 1:116 for mutation *c.943C→T* and 1:260 for mutation *c.918-919insA* based on Hardy-Weinberg proportions. In the 2 987 inhabitants of the village east of Joliette, where we observed the greatest concentration of patients, the carrier frequencies could be as high as 1:18 for the more common *c.943C→T* mutation and 1:28 for the rarer *c.918-919insA* mutation.

The Phenotype of HSAN2 is Relatively Similar Between Carriers of Different Mutations

The clinical, electromyographic and pathologic characteristics of HSAN2 are well established[2, 5]. The sixteen French-Canadian patients present a relatively homogeneous HSAN2 phenotype, as summarized in Table XII (p. 57). The diagnosis of HSAN2 in these families was

made clinically because the mode of transmission was recessive, as opposed to dominant in HSAN1, there was no major autonomic dysfunction as found in HSAN3, the intelligence of patient was normal as opposed to HSAN4 and stretch reflexes were usually absent while they are present in HSAN5 [3, 10, 26, 52, 78]. All were diagnosed before age fourteen (eight months to thirteen years, mean eight years old). Patient 1, at age eight months, was the youngest to be diagnosed. His early neurological evaluation was prompted because his parents already had an older affected daughter. During his first evaluation, he demonstrated diminished sensitivity to pinprick and had no sensory nerve action potential. However, his deep tendon reflexes were present except at the ankles. At age two, he has a history of many painless injuries including wounds that are slow to heal. The two larger genotype groups are the homozygotes for mutations c.943C→T (nine individuals) and compound heterozygotes for both mutations (six patients). There appear to be no striking differences in the age of diagnosis, first infection, and necrosis between these two groups (Table XII p. 57). Furthermore, if one compares the degree of amputations, though there is the expected increase with age, older patients of different genotypes appear equally disabled (Fig. 4 p. 58).

DISCUSSION

This study documents the existence of a large cluster of French-Canadian families with HSAN2. We demonstrate that two ancestral chromosomes carrying two distinct *HSN2* mutations are responsible for HSAN2 in the French-Canadian population. We also describe a novel homozygous nonsense mutation affecting an HSAN2 patient of Lebanese origin different from the one recently uncovered in another HSAN2-affected individual from Lebanon [119]. The higher prevalence of two *HSN2* mutations in Québec appears to be responsible for the fact that more than 50% of patients described in the literature prior to this study were of French-Canadian background. This study suggests that the Lanaudière region of southern Québec

province may have a much higher prevalence of this disease than other regions in the world. The higher prevalence of HSAN2 in this region is due to higher carrier frequencies for both French-Canadian mutations but in particular for the apparently rarer *c.918-919insA* mutation. In fact, 58% (7/12) of patients in this region are compound heterozygotes. Furthermore, a higher carrier frequency for mutation *c.918-919insA* in the small village with affected individuals in five families (A, E, F, G and K) is probably responsible for this unfortunately higher local prevalence of the disease. The estimated combined frequencies for both mutations in the Joliette county based on the observed number of homozygotes for either mutation is in the order 1:80, whereas it is 1:11 in the small village east of Joliette with the greater number of patients. The overall regional carrier frequencies are smaller than the ones for more studied Québec recessive mutations found in the northeastern region of Saguenay-Lac-Saint-Jean, such as: 1:22 for autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS, OMIM 270550) and 1:23 for hereditary motor and sensory neuropathy with agenesis of the corpus callosum (HMSN/ACC, OMIM 218000) [122]. However, in the small Lanaudière village, the individual mutation frequencies are in the same order of magnitude. Moreover, the estimated combined frequency is even higher, further increasing the risk for unrelated parents from the village to have affected children. The relative frequency of the most common mutation is also different between the more common southwestern founder mutation of HSAN2 (75%) and diseases with northeastern Québec founder effects such as ARSACS (94%) or HMSN/ACC (99%) [123, 124]. A population base study is needed to establish more precisely the carrier frequencies in Québec and in the Lanaudière region to determine if a regional base screening program for the *HSN2* mutations should be offered. However, the availability of mutation screening already allows accurate genetic counseling for future parents at risk of being carriers of these mutations.

The genotype-phenotype correlation for French-Canadian HSAN2 carriers of different mutations does not suggest that the two identified mutations influence the severity of the phenotype. Neither was any significant clinical difference observed between the French-

Canadian patients and the individual of Lebanese origin homozygous for a different *HSN2* mutation. This is not entirely surprising considering the nature of the three mutations, all being predicted to lead to the formation of truncated proteins. Future comparisons with the phenotypes of parents with other mutations may help establish if the mutations in French-Canadian patients lead to partial or complete loss of function of the truncated mutated HSN2 proteins. *HSN2* is the fifth gene identified to date to cause a hereditary sensory and autonomic neuropathy. *HSN2* is a novel gene of unknown function. Highly conserved *HSN2* homologues are present in the pig, mouse and rat [118]. Functional bioinformatics analysis identified only a putative *N*-terminal domain signal peptide with a cleavage site that suggests that HSN2 is a secreted protein. Multi-tissue adult northern blot and reverse transcriptase PCR analyses were not able to uncover significant expression in any tissue, suggesting that *HSN2* is probably expressed in a few tissues or at low levels during development. However, the expression of *HSN2* has not yet been studied in fetal tissues. Serial examinations in our cohort demonstrate that the sensory deficit, as documented by sensory levels and loss of deep tendon reflexes, likely progresses with age. The examination of the eight month-old Patient 1 with HSAN2 demonstrates that sensation is likely abnormal from birth, suggesting that HSN2 may play a role in the development of the peripheral sensory system.

Our current knowledge of the mutated genes in HSANs suggests that they are likely involved in different pathways in neuronal development or survival mechanisms. The *SPTLC1* gene (serine palmitoyltransferase long-chain base subunit 1 gene) mutated in dominant HSAN1 plays a role in sphingolipid biosynthesis by catalyzing the pyridoxal-5'-phosphate-dependent condensation of L-serine and palmitoyl-CoA to 3-oxosphinganine [10, 38]. Four studies have suggested that the mutated *SPTLC1* through a dominant negative effect diminishes the function of the normal protein leading to a relative loss of function [38, 39, 45, 125]. The *IKBKAP* gene (IKB kinase complex-associated protein gene) is mutated in recessive HSAN3 [49, 52]. The IKBKAP protein is a scaffold protein important for the assembling of the IKK complex and a regulator for

three kinases involved in pro-inflammatory cytokine signaling [50]. In disease, IKBKAP is absent or dysfunctional. The *TrkA* (tropomyosin-related kinase A) gene mutated in HSAN4 was identified in 1996 using a candidate gene strategy [78]. The *TrkA* gene (1q21-q22) is the human homologue of the neurotrophic tyrosine kinase receptor type 1 gene (*NTRPK1*) deleted in mice insensitive to pain with anhidrosis [61, 64, 63, 71, 80, 81, 82, 83, 84, 126]. It is proposed that the nerve growth factor (NGF)-TrkA system plays a crucial role in the development and function of the nociceptive sensory system and in the establishment of temperature regulation via sweating [71]. The *NGFB* (NGF-β) gene is mutated in HSAN5 [26]. NGF is a polypeptide involved in the regulation of growth and differentiation of certain sensory neurons. NGF is composed of three subunits: α, β and γ, which specifically interact to form a complex [63]. The five mutations described in *HSN2* gene are likely loss-of-function mutations, therefore suggesting that, as in the other HSANs, it is the loss of the protein or inactivation of its function that is responsible for the sensory neuropathy. Further study of the *HSN2* gene may provide important insights into the pathophysiology of these rare neuropathies and the development and preservation of the sensory and autonomic nervous systems.

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Thirteen French-Canadian Hereditary Sensory and Autonomic Neuropathy Type 2 (HSAN2) Pedigrees

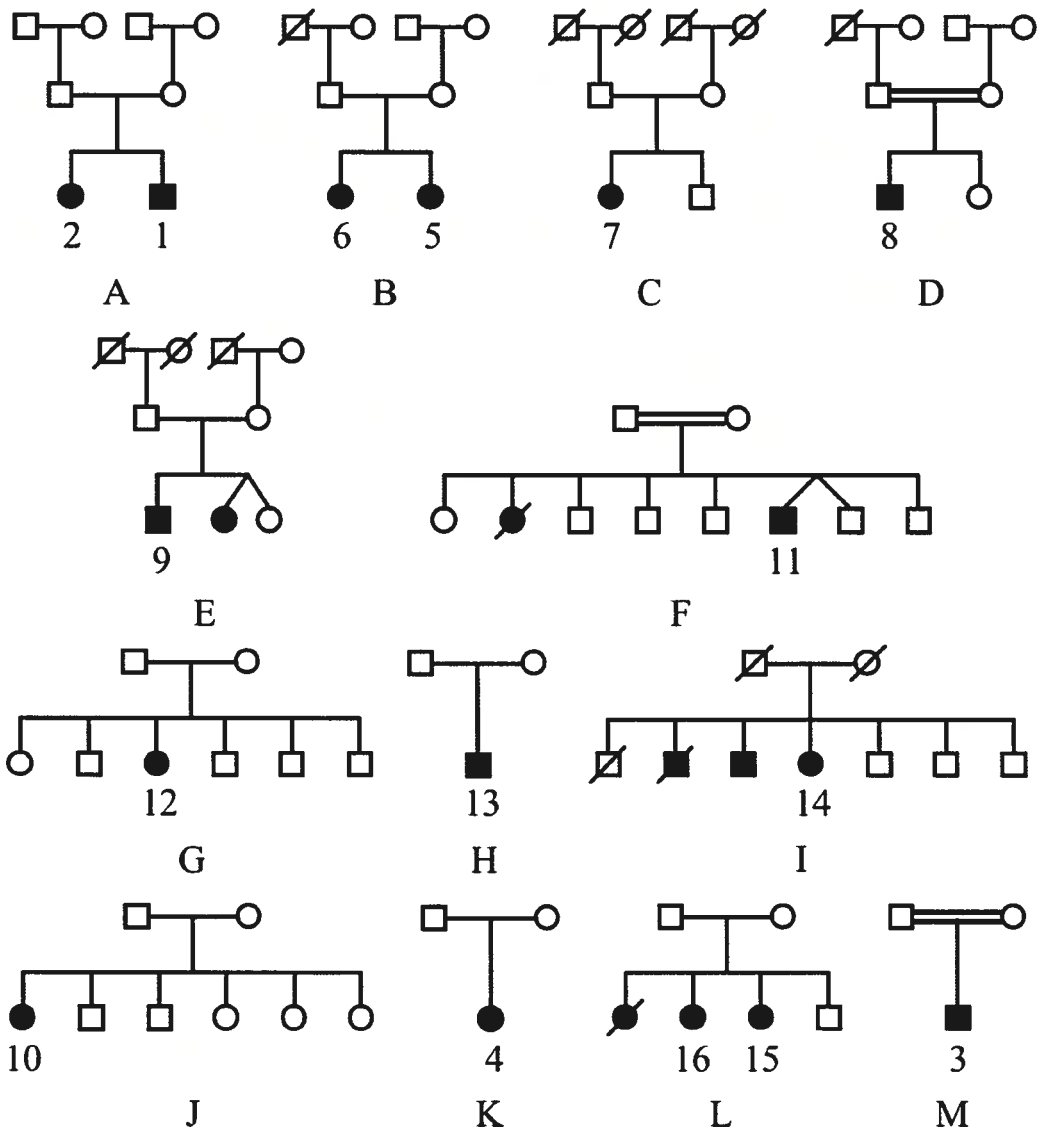

Figure 2. Thirteen French-Canadian Hereditary Sensory and Autonomic Neuropathy Type 2 (HSAN2) Pedigrees. We identified and contacted 18 HSAN2 patients belonging to 13 families living in southern Québec.

French-Canadian Cluster of HSAN2

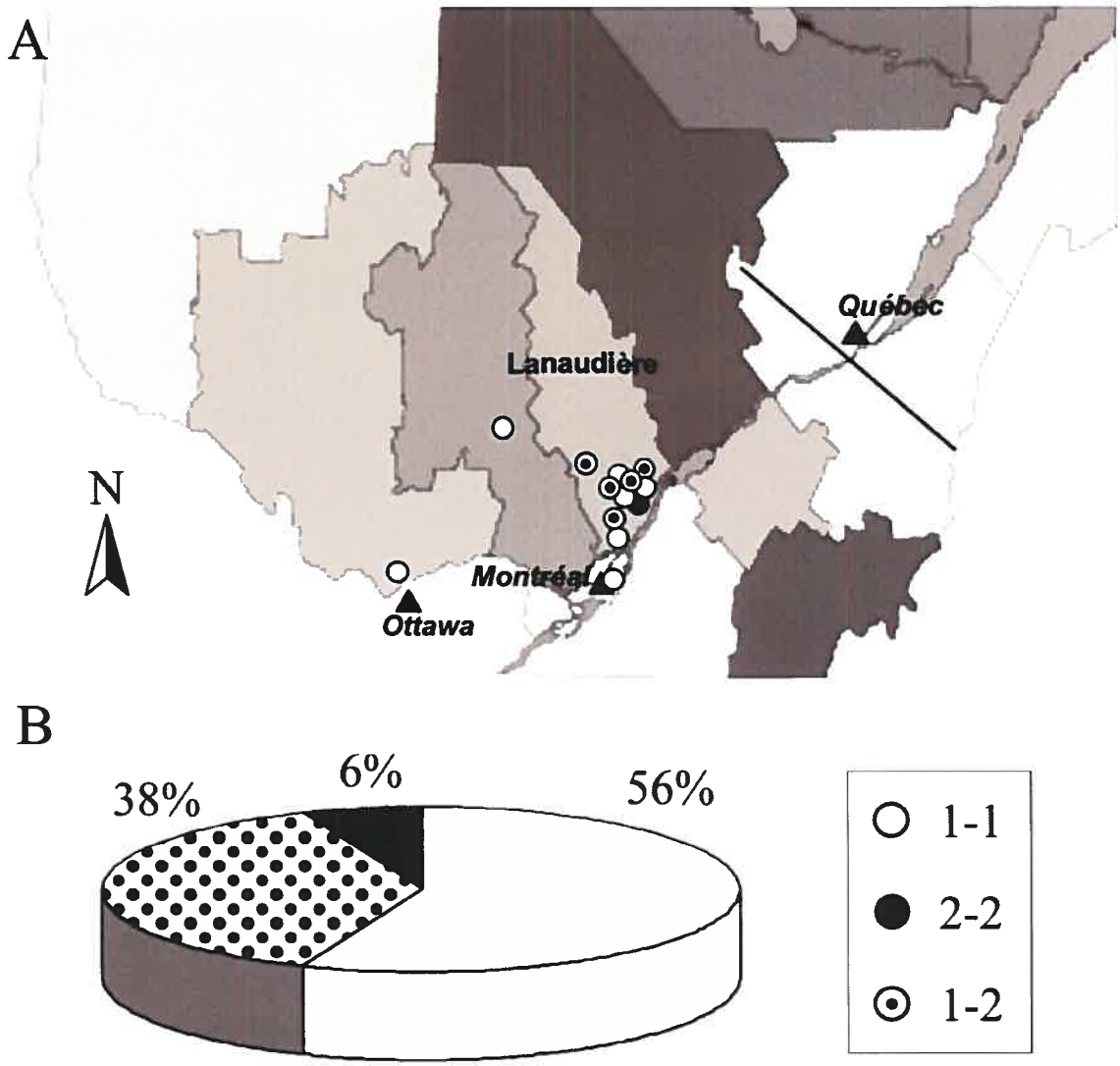

Figure 3. French-Canadian Cluster of HSAN2. Regional origin and mutation distribution for 13 French-Canadian hereditary sensory and autonomic neuropathy type 2 families. (A) Regional origins of patients. Circles represent the place of residence of the patients' parents and the genotype of the affected individuals. White circles stand for homozygotes for mutation *c.943C→T*, black circles for homozygotes for mutation *c.918-919insA*, and dotted circles for compound heterozygotes for both mutations. (B) Percentage of carriers of the two founder mutations. Symbol codes are identical to those in A.

Table XI

Haplotypes of 26* French-Canadian HSAN2 Carrier Chromosomes in the *HSN2* 12q13.33 Region

Markers	D12S352	D12S200	**D12S1455**	D12S91	HSN2	D12S389	**D12S388**	D12S1285	D12S1608	D12S1656	D12S1642	D12S100
Decode (cM)	0	-	-	1.03	-	1.69	-	-	3.65	4.12	-	4.76
UCSC July 2003 (Kbp)	532	652	**711**	817	847	984	**1 099**	1 404	1 629	1 677	1 904	2 047
Cases												
1	2	2	**2**	2	3	2	**3**	5	5	2	1	3
1	3	2	**2**	1	2	1	**4**	5	8	3	7	3
2	2	-	**-**	-	3	-	**3**	5	5	2	1	3
2	3	-	**-**	-	2	-	**4**	5	8	3	7	3
4	3	2	**1**	1	2	1	**4**	5	4	2	4	2
4	2	2	**2**	2	3	2	**3**	5	5	2	1	3
5	4	1	**2**	2	3	2	**3**	5	5	2	1	3
5	3	2	**2**	-	2	1	**4**	5	4	5	7	3
6	4	1	**2**	2	3	2	**3**	5	5	2	1	2
6	3	2	**2**	1	2	1	**4**	5	4	2	7	3
7	2	2	**2**	2	3	2	**3**	4	5	2	1	3
7	2	2	**2**	2	3	2	**3**	4	5	2	1	3
9	2	-	**2**	2	3	2	**3**	5	5	2	1	3
9	2	-	**2**	2	3	2	**3**	5	5	2	1	3
10	2	2	**2**	2	3	2	**3**	5	5	2	1	3
10	2	2	**2**	2	3	2	**3**	5	5	2	1	3
11	3	2	**2**	1	2	1	**4**	5	4	2	4	4
11	3	2	**2**	1	2	1	**4**	5	4	2	4	2
12	2	-	**-**	-	3	-	**3**	5	5	2	1	3
12	2	-	**-**	-	3	-	**3**	5	5	2	1	3
13	2	2	**2**	2	3	2	**3**	5	5	2	1	2
13	2	2	**2**	2	3	2	**5**	5	5	2	7	3
14	4	1	**2**	2	3	2	**3**	5	5	2	1	3
14	3	2	**2**	1	2	1	**4**	5	4	2	4	2
15	-	-	**-**	-	3	-	**-**	5	5	2	1	3
15	-	-	**-**	-	3	-	**-**	5	5	2	3	2

Bold: Flanking markers

* Haplotypes could not be not generated for cases 3, 8 and 16.

DNA was extracted through standard techniques for 16 HSAN2 patients belonging to 13 families. Genotyping was done using standard techniques for 17 markers on chromosome 12q and haplotypes were deduced from family data and generated for 11 of the 17 markers analyzed. Haplotyped carrier chromosomes were studied for all affected patients, except for three individuals for whom no other familial samples were available (patients 3, 8 and the Lebanese patient). DNA sample for patient 16 was too limited to allow full genotyping. In the *HSN2* column, 1 stands for the c.943C→T mutation and 2 for the c.918-919insA mutation.

Table XII

Clinical Phenotype of 16 French-Canadian Patients with HSAN2

No.	Mutation Genotype	Family	Sex	Age at Diagnosis	Age when Recruited	Age at First Infection	Age at First Necrosis	Reflexes	SNAP
1	1-2	A	M	8 mo	8 mo	-	-	Present except Achilles'	-
2	1-2	A	F	4 y	4 y	-	-	-	-
3	1-1	M	M	9 y	9 y	-	-	-	-
4	1-2	K	F	6 y	12 y	6 y (foot)	10 y	Absent except Achilles'	-
5	1-2	B	F	10 y	19 y	10 y (toes)	16 y	-	-
6	1-2	B	F	5 y	22 y	5 y (tibia)	-	Absent except Achilles'	-
7	1-1	C	F	8 y	25 y	7 y (foot)	-	Absent except Achilles'	-
8	1-1	D	M	8 y	28 y	6 y (foot)	14 y	-	-
9	1-1	E	M	11 y	31 y	18 y (foot)	20 y	-	-
10	1-1	J	F	9 y	33 y	5 y (foot)	16 y	Absent except Achilles'	-
11	2-2	F	M	11 y	35 y	12 y (finger)	16 y	-	-
12	1-1	G	F	3 y	43 y	3 y (foot)	7 y	Absent except Achilles'	-
13	1-1	H	M	12 y	44 y	12 y (finger)	10 y	Absent except Achilles'	-
14	1-2	I	F	9 y	58 y	7 y (toe)	11 y	-	-
15	1-1	L	F	10 y	71 y	10 y (foot)	10 y	-	-
16	1-1	L	F	13 y	73 y	7 y (hand)	13 y	-	-
Mean				8 y		8.4 y	13 y		

mo = months; y = years; SNAP = sensory nerve action potential; mutations: 1 = *c.943C→T*; 2 = *c.918-919insA*; (-) = absent

Progression of Amputations in Sixteen HSAN2 Patients According to Age

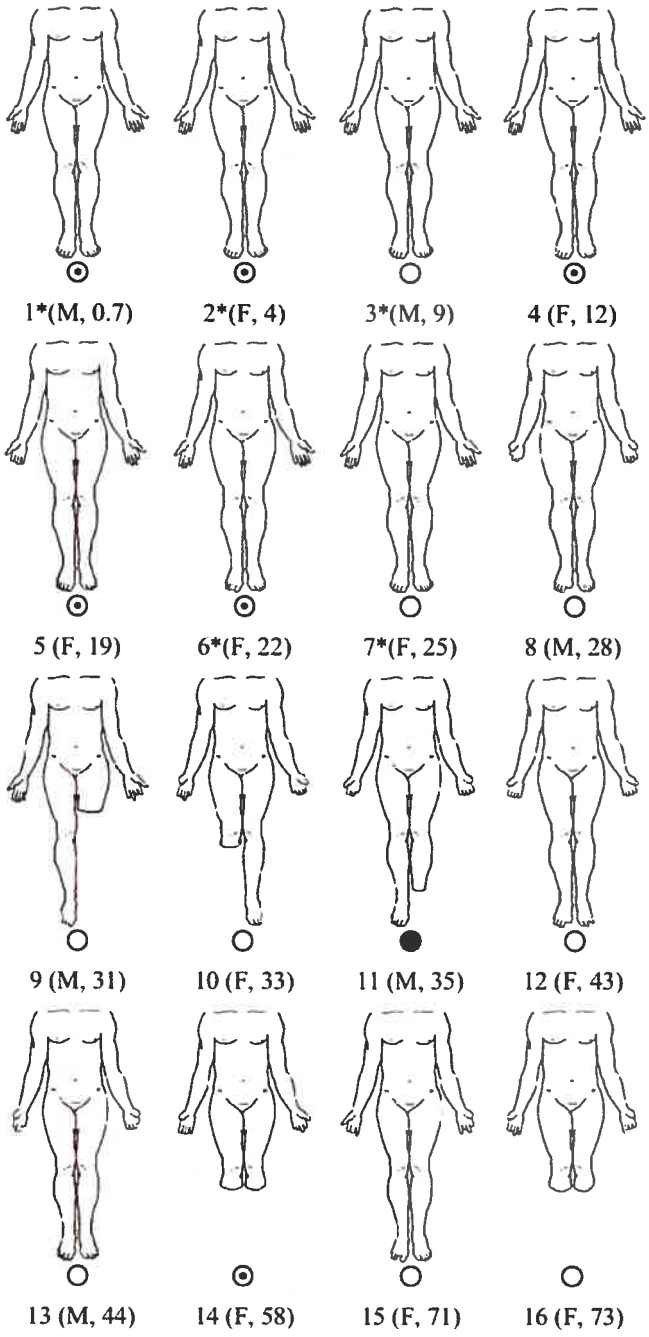

Figure 4. Progression of Amputations in Sixteen HSAN2 Patients According to Age. Genotype and individual legend:

White circles represent homozygotes for mutation c.943C→T
Black circles homozygotes for mutation c.918-919insA
Dotted circles compound heterozygotes for both mutations
Patient number (gender, age at examination)

*Patients 1, 2, 3, 6 and 7 have not been amputated.

DISCUSSION

REVIEW OF THE LITERATURE

A review of the literature on Hereditary Sensory and Autonomic Neuropathy Type 2 at the onset of this study revealed a rare disorder that had been described since the early 1900s. While it was initially confounded with other disorders comprising similar clinical features, the criteria for HSAN2 were clearly established in 1973 by Dyck and Ohta [2]. However, certain aspects of HSAN2 remained unclear. The most important of these were the age of onset of the disease and the question of its clinical progression over time. Furthermore, the molecular basis of HSAN2, including the responsible gene and the pathophysiology of the disease had not been addressed to date.

CHARACTERIZATION OF THE QUÉBEC HSAN2 COHORT

This study was centered about the largest cohort of HSAN2 cases identified to date. The geographical aggregation of the cases discussed here, together with the clear uniformity of phenotype strongly suggested a founder effect for the disorder in the Lanaudière region of Québec. A *founder effect* is defined as a high prevalence of a genetic disorder in a population or its rarity elsewhere [127]. It is believed that the mutation is introduced into the genetic pool of a region by migration of an individual into the group, or by *de novo* mutagenesis. The mutation is then in strong linkage dysequilibrium with adjacent polymorphisms on its harbouring chromosome. Thus, the combination of alleles at this site, also known as the *founder haplotype*, becomes the so-called *genetic signature* for this effect (and for the founder chromosome) [127]. Subsequent demographic expansion in an initially small population establishes the high frequency of the founder haplotype in the population and thus stabilizes the

founder effect [127]. Such founder effects have been previously shown for other rare hereditary diseases in northeastern Québec, including autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS), hereditary motor and sensory neuropathy with agenesis of the corpus callosum (HMSN/ACC) and pseudo-vitamin D-deficiency rickets [122, 123, 124, 127, 128, 129, 130].

Our group was evaluated in detail using the established criteria, in order to ensure that they in fact all suffered from the same disease. An analysis of the clinical features of this cohort along its full age spectrum indicated that the disease has a biphasic course (see Addendum). Progressive sensory neurological deficit and loss of deep tendon reflexes, which begins in childhood, continues throughout adolescence but tends to stabilize in young adulthood after the achievement of maximal limb length. Acral mutilations and amputations also follow a progressive course, with subjective stabilization in adulthood.

MOLECULAR STUDIES

The first step in our genetic study of HSAN2 was to exclude that this disorder was linked to any of the previously identified loci for HSANs types 1, 3 and 4. Subsequently, the *HSN2* gene was identified on chromosome 12q13.33 [118, 119]. This gene consists of a single (approximately 3 kilobase) exon located within intron 8 of the *PRKWNK1* (protein kinase, lysine deficient 1) gene [118]. Its product is a novel protein, predicted to contain 434 amino acids, that has no sequence similarity with any known protein. Its function is not yet known, but analysis of its amino acid sequence suggests that it may be a secreted protein and thus may potentially represent a not yet identified neurotrophic factor [118]. Sequencing of the *HSN2* gene in our cohort identified two mutations among the French-Canadian group and a novel misssense mutation in another patient of Lebanese origin [90]. The estimated carrier frequencies were compatible with a regional founder effect for the disease [90]. The five mutations described to date in HSAN2 cohorts (including four previously identified mutations and an additional

mutation sequenced in the present cohort), have all been predicted to cause truncation of the HSN2 protein, leading to loss of function. Clinical comparison of patients with different mutations has confirmed the phenotypic homogeneity.

PROPOSED PATHOPHYSIOLOGIC MECHANISMS

Classification of HSAN2 Among the Peripheral Neuropathies

Peripheral neuropathies have been classified into three large groups: *neuronopathies*, where the primary pathological process resides in the cell body; *myelinopathies*, which consist of primary myelin disease; and *axonopathies*, which affect the distal parts of the longest axons [131]. The axonopathies are the most common type, where the objective clinical deficit is the resultant of distal degeneration minus the regeneration of axon tips [131]. HSAN2 represents a hereditary axonopathy, whereas well-known acquired axonopathies include the diabetic, HIV-related and chemotherapy-induced sensory neuropathies.

The Role of Retrograde Transport in the Peripheral Axonopathies

The function of the novel HSN2 protein has not yet been elucidated as noted above. Thus at present, conceivable mechanisms for the neuronal loss seen in HSAN2 are manifold. Any of the systems involved in neuronal growth and survival could be implicated, as may be deduced from the study of the other forms of HSAN. HSAN1 has been attributed to decreased enzymatic function of serine palmitoyltransferase (SPT). HSAN3 patients have absent or dysfunctional IKB kinase complex-associated protein (IKAP). HSANs 4 and 5 are the results of defects in the nerve growth factor/tropomyosin-related kinase A (NGF-TrkA) system. A recent article suggested that altered vesicular transport in lengthy neurons may be a common pathophysiologic mechanism in all the HSANs [9]. This was inferred from detailed studies of the known protein products of the other HSAN loci. SPT is involved in endocytic membrane

trafficking events, IKAP is involved in late stages of vesicular trafficking, and the NGF-TrkA complex must be transported in signalling endosomes along peripheral nerve axons to the cell bodies of nociceptive neurons [9, 15, 42, 59, 132, 133]. Retrograde transport of Trks from axon to soma is vital for the survival of target-derived neurotrophin (NT)-dependent neurons. This was demonstrated by experimental inhibition of dyenin-dependent rapid retrograde Trk transport, which resulted in degeneration of these neurons [134]. Absent or decreased retrograde transport deprives the soma of signalling molecules and other proteins from the axon terminal. As a result, the cell body cannot respond appropriately to repair axonal damage, even if surrounding cells (e.g. Schwann cells) begin to mount a response [66]. Impaired retrograde transport seems to be a logical general mechanism for HSAN2, given the typical length-dependent, "distal-first" involvement [4, 87, 135].

Possible Functions of the HSN2 Protein

The five known mutations in the *HSN2* gene are thought to be loss-of-function mutations, resulting in loss or inactivation of its protein product. To date, northern blot and rtPCR studies in adult tissues have not demonstrated significant expression of the HSN2 protein in any tissue, which suggests that it is either expressed in few tissues, or possibly only during development [90]. Its physiologic role is difficult to surmise, but if it is indeed a secreted protein, it may possess neurotrophic actions. If not, it may function as an adaptor molecule coupled to neurotrophin receptors for intracellular signal transduction, play another role in trophic signal transduction cascades or even act as a transcription factor. Future studies will be necessary to help elucidate this role, including further analysis of HSN2 expression in normal as well as in pathological tissues.

Parallels Between HSAN2 and Diabetic Sensory Neuropathy

A closer look at the pathophysiologic basis of diabetic neuropathy may provide some clues as to the cause of HSAN2. The progressive distal symmetric sensory loss seen in diabetic neuropathy resembles that of HSAN2 and these patients also present impaired skin healing, with eventual complications of trophic skin changes, nonhealing ulcers, tissue necrosis, gangrene and limb amputation. Peripheral neuropathy (initially affecting nociceptive and proprioceptive sensory neurons and eventually motor and visceral autonomic neurons) is the most common complication of diabetes mellitus, affecting over 50% of all patients, and accounting for over 60% of all lower extremity amputations in the general population [66, 136]. Furthermore, damage to cardiac autonomic innervation (thinly myelinated Aδ- and unmyelinated C-fibers) leads to the loss of pain perception of myocardial ischemia [137]. Molecular studies of diabetic neuropathy have implicated many pathogenic factors including polyol pathway activation, advanced glycosylation end products, vascular insufficiency, neuronal membrane ion channel dysfunction and (potentially more relevant to HSAN2) deficiency of neurotrophic factors [138].

Function of the Neurotrophin System

The neurotrophin (NT) family consists of four members: NGF, brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5), which have approximately 50% sequence homology, but bind to distinct targets via specific receptors [66, 139]. Trk receptors (Trks A, B and C; members of the tyrosine kinase family of receptors) are the specific NT receptors, which bind their preferred substrates with high affinity. NT binding to Trk receptors promotes survival through activation of the P13K/Akt cascade as well as through phosphorylation and inactivation of proapoptotic substrates such as Bad. It also promotes cell differentiation through activation of the Ras/MAPK, P13K and PLC cascades [139, 140]. The p75 neurotrophin receptor ($p75^{NTR}$ or simply p75; a member of the tumor necrosis factor family of

receptors) binds all of the neurotrophins with low affinity and it is thought that it can either direct them to the high-affinity Trk receptors for promotion of trophic intracellular signalling cascades (thus enhancing Trk response to preferred ligands), or conversely in the absence of Trk receptors promote apoptosis [66, 85, 133, 141, 142, 143, 144, 145]. The actual preferred ligands of the p75 receptor in the presence of another receptor known as *sortilin* are the *proneurotrophins*, which also favor apoptosis and growth inhibition through activation of the JNK/p53/Bax proapoptotic pathway [140, 146, 147]. Thus, NGF can have opposing physiological effects; the overall effect being modulated by differential activation of TrkA, p75 and sortilin receptors.

The intra-neuronal trophic actions of the NTs can be classified into three categories based on their spatial location. First, NT-receptor binding and local signalling at the axon terminal leads to neurite outgrowth and remodelling. Second, the NT-receptor complex is incorporated into endosomes, which are transported towards the cell body through retrograde axonal transport [66, 148]. Here, sustained signalling is made possible by maintenance of the tyrosine kinase domains on the outer surface of the endosomes, facing the cytoplasm. Finally, in the cell soma, NTs regulate transcription in order to alter overall cellular structure and function [144, 147, 149].

The NTs have two major roles in the development and maintenance of neurons. The first *trophic* role, promotes their survival and/or growth. The second *tropic* role, directs the movement of extending neurites towards their appropriate targets. Together, these functions allow for the formation and maintenance of appropriately connected neurons [66]. Different NTs and their respective receptors are involved in the support of different neuron subpopulations [139]. As mentioned previously, the NGF/TrkA system is essential for the development of sympathetic and neural-crest-derived dorsal root ganglion (DRG) nociceptive neurons [68]. Most small-caliber cutaneous sensory fibers are nociceptors, which originate from TrkA-expressing neurons innervating laminae I and II of the spinal cord dorsal horn [68]. NGF is synthesized by target organs and by Schwann cells associated with these nerve fibers during development. TrkA

receptors are also highly expressed in the DRG during this period [66]. The *neurotrophic hypothesis* suggests that competition for a limited supply of neurotrophic factors determines the survival of neurons during embryologic development, in order to appropriately tailor the number of innervating neurons to the target tissue [66, 141, 142]. This same mechanism may also have a role in the regeneration of damaged neurons in normal "wear-and-tear" or in disease states. Adult peripheral neurons regain growth ability after injury; thus the availability of neurotrophic factors may be key for the survival of these damaged neurons [66, 142]. In support of this theory, it has been shown that NGF and TrkA are upregulated in the L4 and L5 DRG after sciatic nerve crush injury [66, 150]. In addition, NGF, TrkA and p75 are all increased in Schwann cells distal to sciatic nerve injury. Furthermore, levels decrease with progressive regeneration and contact of these axons with the Schwann cells [66, 142].

Extra-neuronal Actions of Neurotrophins

It is known that NT effects are not limited to neurons [151, 152]. Interestingly, the common ectodermal embryologic origin of the nervous and epithelial systems suggested the implication of common growth factors in neural and skin homeostasis and remodelling [151, 153]. In reality, the NTs are multifunctional growth factors that also have effects on Schwann cells, immune cells (lymphocytes, mast cells and eosinophils) and skin cells (keratinocytes, melanocytes and fibroblasts) [152, 153].

It is known that NTs are expressed in embryonic mouse skin. Postnatal proliferating (but not mature differentiated) skin basal keratinocytes synthesize and release NGF [151]. NTs are produced by fibroblasts *in vitro* and NGF is known to stimulate fibroblast migration and tissue remodelling. All four NT receptors including TrkA and p75 are found in human epidermal keratinocytes. Studies have shown that inhibition of TrkA phosphorylation blocks keratinocyte proliferation in the absence of exogenous NGF [151]. Furthermore, human keratinocytes transfected with NGF have increased proliferation rates compared to mock-transfected cells and

keratinocytes overexpressing TrkA also proliferate better than controls [151]. Apoptosis is known to be an important mechanism in skin homeostasis, which counterbalances proliferation; thus there is presence of apoptotic cells in normal human epidermis. NGF rescues human epidermal keratinocytes from spontaneous UVB-induced apoptosis *in vitro* through activation of the Bcl-2 family of anti-apoptotic proteins, consequently blocking caspase activation [151].

NGF and TrkA are also expressed in immune cells of normal skin including mast cells, macrophages, lymphocytes and endothelial cells [151]. It is thought that the NTs play an immunomodulatory role in normal skin as well as in pathological conditions including wound healing and inflammation through their effects on these cells [151].

The association of peripheral nerve and epidermal anomalies in both diabetic neuropathy and in HSAN2 is intriguing, and may argue in favor of involvement of the NT systems in the pathophysiology of both of these diseases.

The Role of NGF in Diabetic Sensory Neuropathy

Basic studies of the NGF system in diabetes models demonstrate decreased baseline NGF expression in skin and muscles, decreased TrkA and p75 expression in DRGs, as well as decreased NGF peak expression after nerve injury [136, 138, 154, 155]. In addition, decreased retrograde transport in the sciatic nerve has been demonstrated, leading to decreased NGF effects in the cell body [155, 156]. NGF supplementation has improved diabetic neuropathy in animal models, as evidenced by decreased hypoalgesia, improved SNAPs in the feet, upregulation of substance P and calcitonin gene-related peptide (CGRP) (both NGF-dependent neuropeptides) in the DRG, and regeneration of neuronal fibers [66, 137, 156, 157]. In addition, topical NGF application accelerates wound healing in diabetic mice [158, 159]. As an alternative to exogenous neurotrophin administration, CB 1093 (a vitamin D3 derivative), enhances NGF levels in tissues. It also maintains levels of substance P and CGRP in the small sensory fibers of diabetic rats [155]. Similarly, the small-molecule hypoxanthine derivative, Neotrofin, increases

NGF levels in the DRG of adult rats and promotes collateral sprouting of NGF-dependent peripheral nerves in the skin [155]. Retinoic acid is another compound that has been shown to increase serum and nerve NGF levels in experimental animals [155, 157].

The success of NGF administration in animal diabetic neuropathy models has led to human clinical trials. Phase 2 placebo-controlled clinical trials of subcutaneous recombinant human NGF (rhNGF) administration demonstrated subjective improvement after six months [136]. However, phase 3 double-blind placebo-controlled trials did not confirm this effect. This has been attributed to several factors including suboptimal delivery methods, dose limitation by local pain at the injection site and possible induction of anti-NGF antibodies [136, 137]. These potential difficulties with NGF administration may be overcome in future trials with other compounds that enhance NGF effects, such as in the animal studies mentioned above [154]. These findings may eventually be extrapolated to HSAN2 patients.

FUTURE DIRECTIONS

Genetic Counselling for HSAN2

The discovery of the gene responsible for HSAN2 will eventually permit genetic counselling for couples in target regions with high carrier-rates for the *HSN2* gene. These individuals are at risk of passing this severely morbid disorder with no known treatment to their offspring [160]. This type of commercial testing is already available for HSANs 1 and 3, and is done for free in Dr. B. Brais' laboratory for Québec HSAN2 cases [13, 161].

Therapeutic Strategies for HSAN2

Further study of the expression and function of the HSN2 protein should shed a better light on this fascinating disorder of the peripheral nervous system. It may also increase our

knowledge about the development and maintenance of the somatosensory and autonomic systems and the pathophysiology of neuronal death.

These types of molecular studies may allow for further advances in the treatment of patients with HSAN2 and the other hereditary neuropathies, as well as patients with acquired forms of peripheral sensory neuropathy (including the diabetic and iatrogenic neuropathies). Potential strategies include methods of decreasing axonal degeneration or increasing regeneration. Options include targeted therapies aimed towards the specific molecular defect responsible for the disease process (i.e. the mutated HSN2 protein). Studies are already underway for such targeted therapies in HSAN3, where various molecules have been studied in an effort to increase the production of wild-type IKAP protein as opposed to the mutant splice variant [9, 55, 162, 163, 164]. Other more general strategies may target axonal growth and regeneration pathways, including the transcription factors that regulate these processes, the genes activated by these transcription factors, and the neurotrophins such as NGF (as has been attempted for diabetic neuropathy). One or a combination of these methods may one day result in effective treatment for patients suffering from HSAN2.

CONCLUSION

A detailed review of the literature on Hereditary Sensory and Autonomic Neuropathy Type 2 was performed at the onset of this study. This rare disorder has been described since the beginning of the 21st century and was initially confounded with other disorders comprising similar clinical features. Specific criteria for HSAN2 were established in 1973 by Dyck and Ohta [2]. Nevertheless, certain aspects of HSAN2 remained unclear. With the discovery of the largest cohort of HSAN2 cases identified to date, one of our goals was to elucidate the age of onset of the disease and the question of its progression over time. Furthermore, recent advances in molecular biology opened the door for our quest to define the molecular basis of HSAN2, including the responsible gene and the specific consequences of its mutations.

Clinical phenotype was evaluated in detail for the whole group, in order to ensure that all cases suffered from the same disorder. Genealogic study confirmed a transmission pattern compatible with an autosomal recessive inheritance, which had already been established for HSAN2. The geographical aggregation of the cases, together with the marked uniformity of phenotype strongly suggested a founder effect for the disorder in the Lanaudière region of Québec. An analysis of clinical features of this cohort along a large age spectrum indicated that the disease has a biphasic course where progressive sensory neurological deficit and loss of deep tendon reflexes, which begins in childhood, continues throughout adolescence and limb growth, but tends to stabilize in young adulthood.

In the initial phase of the genetic study, we excluded the possibility that this disorder was linked to any of the previously identified loci for HSANs types 1, 3 and 4. Subsequently, the *HSN2* gene was identified on chromosome 12q13.33 [118, 119]. *HSN2* consists of a single (approximately 3 kilobase) exon located within intron 8 of the *PRKWNK1* (protein kinase, lysine

deficient 1) gene [118]. The protein product is predicted to contain 434 amino acids and has no sequence similarity with any known protein. Its function is not yet known, but analysis of its amino acid sequence suggests that it may be a secreted protein [118]. Sequencing of the *HSN2* gene in this group identified two mutations among the French-Canadian group and a novel misssense mutation in another patient of Lebanese origin. The five mutations described to date in HSAN2 cohorts (including four previously identified mutations and the new mutation sequenced in the present cohort), have all been predicted to cause truncation of the HSN2 protein. It is thought that these truncations lead to loss of function. Clinical comparison between patients with different mutations has confirmed that the phenotype remains the same despite the various mutations.

The possible mechanisms of the neuronal loss seen in HSAN2 are varied, since the function of the HSN2 protein remains unknown. They could implicate any of the varied systems involved in neuronal growth and survival, as may be inferred from the study of the other forms of HSAN. What distinguishes HSAN2 from the others, however, is that this disorder seems to be progressive and to selectively affect long axons. This leads us to propose two general theories for the pathophysiology of this axonopathy. First, it is possible that the neuronal soma can only maintain an axon of limited length or volume. Alternatively, axonal pathological processes that initiate the process of cell death may act in a manner that is proportional to length or volume. It has been postulated that the normal HSN2 protein is involved in the development or in the maintenance of peripheral sensory neurons or of their associated Schwann cells [118, 119]. Loss of HSN2 function in these cases would lead to early-onset but progressive axonal loss and demyelination in sensory neurons, producing the typical clinical tableau [118, 119]. A recent article has proposed that altered vesicular transport in these lengthy neurons may be a common pathophysiologic mechanism for all the HSANs [9]. This was deduced from detailed studies of the protein products of the other HSAN loci. SPT (the gene

product implicated in HSAN1) is involved in endocytic membrane trafficking events, IKAP (the gene product of the HSAN3 gene) is involved in late stages of vesicular trafficking, and the NGF-TrkA signalling complex (likely involved in HSANs 4 and 5) must be transported in signalling endosomes along peripheral nerve axons to the cell bodies of nociceptive neurons [9, 15, 42, 59, 132, 133]. This theory seems to be a logical general mechanism for these diseases, considering the typical "distal-first" involvement [4, 87, 135].

The discovery of the gene responsible for HSAN2 will eventually permit testing and genetic counselling for couples in target regions with high carrier-rates for the *HSN2* gene, as was the desire of the first patient who presented to our clinic. These individuals are at risk of transmitting a severely morbid disorder with no known treatment to their offspring [160]. This type of testing is presently commercially available for HSANs 1 and 3, and is done for free in Dr. B. Brais' laboratory for Québec HSAN2 cases [13, 161].

The continued study of HSAN2 and of the other HSANs will open the door for a deeper understanding of the development and maintenance of the somatosensory and autonomic systems, as well as the pathophysiology of neuronal death. This could be of interest not only for the HSANs, but for many other neurodegenerative diseases as well.

Further study of the expression and function of the novel HSN2 protein should enhance our knowledge of this fascinating disorder of the peripheral nervous system. These types of molecular studies may allow for further advances in the treatment of patients with HSAN2 and the other hereditary neuropathies. They may also contribute to the treatment of patients with acquired forms of peripheral sensory neuropathy including diabetic and iatrogenic (for example, chemotherapy- or antiretroviral-induced) neuropathies, which may have common pathophysiologic bases. Future treatments potentially include targeted therapies aimed towards the specific molecular defects responsible for the disease process. Studies are already underway for targeted therapies of this sort in HSAN3, where molecules have been studied in

an effort to increase the production of wild-type IKAP protein relative to the mutant splice variant [9, 55, 162, 163, 164]. The same type of strategy may hopefully one day result in effective treatment for patients suffering from HSAN2.

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ADDENDUM: FURTHER CHARACTERIZATION OF THE DISEASE

Participants in this study were questioned and examined, as stated earlier, with two main objectives. The first was to assure the homogeneity of phenotype among the identified cases, in order to clinically confirm that they all suffered from the same disease. The second goal was to address the question of progression of disease.

HOMOGENEITY OF PHENOTYPE

The epidemiologic features of the initial Québec HSAN2 cohort at the onset of our study are summarized in Table XIII (p. xxiii) (Two patients were added to the study after the participation of the author of this thesis, including one French-Canadian patient and one of Lebanese origin, and these were not included in the present analysis). There were ten females and five males from ten different French-Canadian families (33% male, in contrast to the male preponderance observed in the literature (see Chapter 1). The ages of the subjects at examination ranged from 0.7 to 73 years, with an average age of 33 years. There was a history of consanguinity (strictly defined as a first degree relationship between parents) in two families. The mode of transmission was compatible with an autosomal recessive pattern in all of the families, as verified by genealogic reconstructions. However, statistically, our cohort demonstrated an average of 57% of affected siblings, which is much higher than the 25% expected for an autosomal recessive pattern of inheritance. This may be explained in part by the reluctance of certain parents of affected individuals to continue having children.

Historical characteristics (Table XIV, p. xxiv) showed an average age at diagnosis of eight years, with all patients being diagnosed before the age of thirteen. The youngest case in our cohort was diagnosed at the age of eight months, shortly after his sister was diagnosed. He presented with a painless burn of his hand. The age at which the sensory deficit was first noted

in the other patients was variable, and often not recalled by the individual. In fact, many patients claimed that they had always had the same sensory deficits. The onset of severe complications was documented by onset of soft tissue infections, fractures, and of either spontaneous necrosis of limb parts or surgical amputation. First episodes of soft tissue infection occurred between three and twelve years of age, with an average age of eight years. Average age at first fracture was ten years, which was in general later than the first manifestations of the disease. First necrosis and amputation were on average at thirteen and seventeen years, respectively. The younger patients in our study tended to have much less mutilation of the extremities than the patients over the age of 25. Patients had uniform absence of cranial nerve dysfunction (including diplopia and deafness), motor weakness, lightning pains, and autonomic disturbances including postural hypotension, sphincter disturbance or alacrima.

The clinical examination (Table XV, p. xxv) consisted first of inspection of cutaneous lesions and of the extent of amputations in both the upper and lower extremities (Fig. 5, p. xxvi). A sensory exam consisting of tactile and pinprick sensation in upper limbs, face and trunk only was performed. Lower limb sensation was not tested because many patients had severe lesions or amputations at this level. The results of the sensory examination are discussed further in the next section. Extraocular muscle function and sensory trigeminal nerve function were normal in all cases. Muscle strength as manifested by deltoid muscle testing was also normal in all patients. Upper limb deep tendon reflexes were absent or decreased in all patients except the youngest (case number one). The patellar and Achilles' reflexes were absent in all cases in which they could be tested (lower limb amputations precluded testing in some cases), the exception again being the youngest case who lacked only the Achilles' reflex.

Electrophysiologic studies demonstrated absence of an ulnar nerve SNAP (sensory nerve action potential) in all cases including the youngest possible case (case number one) (Table XV, p. xxv).

Pathologic examinations (Table XVI, p. xxvii) were available for four cases only. Case four's sural nerve biopsy was judged as being compatible with HSAN2 according to notes in the medical chart, but the official pathology report was not available. Case seven's sural nerve biopsy demonstrated changes compatible with primary axonal degeneration, with reactive Schwann cell changes and fibrosis. This patient also had a muscle biopsy, which was compatible with changes secondary to the axonal degeneration. Case nine demonstrated complete loss of myelinated fibers in the sural nerve biopsied, with slightly increased numbers of normal unmyelinated fibers. Her tibialis anterior biopsy was similar, but there was presence of some normal myelinated fibers. Case ten showed total loss of myelinated fibers in his sural nerve, along with severe loss of unmyelinated fibers. Residual unmyelinated fibers appeared normal. There were secondary Schwann cell changes, and no evidence of regenerative phenomena.

EVIDENCE FOR PROGRESSION OF DISEASE

Study of the literature on HSAN2 reveals a lack of consensus regarding the presence or absence of progressive neurological deficit. Peter Dyck, in his chapter on the hereditary sensory neuropathies in *Peripheral Neuropathy*, concludes that "there is no unequivocal evidence of clinical worsening" [3]. On the other hand, our group of patients from the Lanaudière region seems to argue in favour of progression, at least during the early phase of the disease.

The historical data collected from our cohort gives several indications of a progressive disease. The most convincing evidence was obtained from the medical charts, where serial neurologic examinations documented a progression of sensory deficits and a loss of deep tendon reflexes with age (Table XVII, p. xxviii). Case number four had sensory deficits extending to wrist- and to knee-levels at age ten, according to chart data, but had clear

hypoesthesia to elbows on our examination at nineteen years of age. Case number five showed progression of sensory deficit between the ages of nine to ten, with levels progressing from a glove-and-stocking distribution to mid-arm and mid-thigh levels. Her deep tendon reflexes were noted to be weak at five years, but were clearly absent on our examination at 22 years of age. Case number seven had hypoesthesia of the feet only at six years of age, but a level at mid-leg on our examination at 28 years. He had weak deep tendon reflexes at six years, which were noted as abolished at thirteen years. Case number eight had presence of all deep tendon reflexes at five years, but they were absent at the age of 31. Case number nine had hypoesthesia below wrists at eleven years, which had progressed to elbow-level at 33 years of age. She had been noted to have weak deep tendon reflexes on the right at eleven years, but was completely areflexic on our examination. Case number ten had hypoesthesia to the elbow at fifteen years, which had progressed to mid-arm level on our examination at 35 years. Case number thirteen was described in Heller and Robb's article as having hypoesthesia of "the periphery of all four limbs, with fading borders to the shoulders and thighs" at the age of nine [35]. On our examination at the age of 58, she had severe hypoesthesia to shoulder level. It is notable that our youngest case at eight months of age (case number one), had preservation of *all* deep tendon reflexes except the Achilles'.

The increased level of amputations with age may also be an indicator of disease progression, though it may only be the additive effects of trauma with aging. On the other hand, many of the older patients also had the subjective impression that soft tissue infections and amputations tended to wane in adulthood, which indicates the possibility of a stabilization of disease after the growth phase.

We obtained quantitative data regarding the extent of absolute and relative sensory deficit in the upper limbs of all patients (except case number one, who was too young for a detailed sensory examination to be performed), with the intention of comparing patients at

different points in the age spectrum and to study the correlation of the deficit with age and gender.

DESCRIPTIVE STATISTICS

The average age at onset of disease, as defined by the initial detection of sensory deficit, first soft tissue infection, first necrosis or first amputation, was seven years (range 0.7-11 years), which is similar to that observed in the literature. There was no significant difference in age of onset in females as compared to males. The average duration of disease for the whole group was 27 years; however, an average duration of thirty years in females as compared to twenty years in males reflects a longer period of follow-up for the females in this cohort. Average ages at first infection were seven years for females, and twelve years for males (Student's t-test, two-tailed; $p = 0.02$); for amputation they were thirteen and 22 years, respectively (Student's t-test, two-tailed; $p = 0.01$), indicating that men tend to have severe complications at an older age.

QUANTIFICATION OF PROGRESSION

"Axon length of the upper limb" was approximated as the distance in centimetres from the spinous process of the C7 vertebra to the ulnar styloid process of each patient (since many had amputations of digits). "Level of anesthesia" as well as "level of hypoesthesia" to tact and pinprick were measured from the wrist proximally in centimetres (Tables XVIII and XIX, p. xxix). "Residual tact and pinprick sensation" was defined as the distance from the C7 spinous process to the proximal limit of absolute anesthesia.

The first hypothesis was that absolute sensory deficits progressed with growth and lengthening of the extremities. The same analyses were then repeated for residual sensory

function in order to test the second hypothesis that the deficit could be related to incapacity of the neuron cell bodies to maintain axonal integrity beyond a certain distance as extremities lengthen. Finally, analyses were done to compare disease progression between the genders.

RESULTS

Statistical analyses were performed using Statistical Package for the Social Sciences for Windows 9.0 (SPSS Inc., Chicago, IL). Average axonal length was 72 centimetres in females compared to 84 centimetres in males (Student's t-test, two-tailed; $p = 0.01$). Anesthesia to tactile stimulation was more extensive than anesthesia to pinprick in only two of the fourteen tested cases (cases six and seven; Fig. 6, p. xxx)

There was a tendency towards a logarithmic relationship between level of anesthesia to tactile stimulation and age (Fig. 7, p. xxxi), as well as between level of anesthesia to pinprick stimulation and age (Fig. 8, p. xxxii). Both of these curves showed a tendency to plateau at approximately twenty years of age, which suggests that the progression of sensory deficits does in fact tend to wane with age. This may explain why some authors failed to demonstrate any progression.

There was a significant positive linear correlation between anesthesia to tactile stimulation and axonal length (Pearson correlation test, two-tailed; $p = 0.01$) (Fig. 9, p. xxxiii) and between anesthesia to pinprick stimulation and axonal length (Pearson correlation test, two-tailed; $p = 0.02$) (Fig. 10, p. xxxiv). These correlations demonstrate that these deficits are directly proportional to arm (axonal) length.

Correlation of residual pinprick sensation with age tended to approximate a linear relationship with a slope approaching zero (slope = -0.02) (Fig. 11, p. xxxv), as did the correlation between residual pinprick sensation and axonal length (slope = 0.3) (Fig. 12, p.

xxxvi). This suggests a critical length for efficient axon maintenance, although these values were not found to be statistically significant.

There was no relationship demonstrated between age at disease onset and residual sensation.

Gender Comparisons

There was no significant difference in the age of onset between females (average = 6 years) and males (average = 8 years).

The absolute extent of anesthesia to pinprick was six centimetres in females, compared to fifteen centimetres in males, but this difference was not statistically significant. In addition, males were older and had longer arm lengths than females, which would naturally lead one to expect a greater absolute length of sensory deficit.

There was no significant difference in residual tactile sensation between females and males (66 versus 69 centimetres).

DISCUSSION

The homogeneity of phenotype demonstrated in our cohort of HSAN2 cases from southern Québec strongly suggested a founder effect for the disease in this area. We now know that these patients all harbor two mutations inherited from shared ancestors who introduced the disease to the area many centuries ago (see Chapter 2).

Analysis of our cohort strongly suggests a progression of disease with age until adulthood, as well as a greater deficit that is roughly proportional to the length of the extremities. In fact, it appears that sensory deficits progress until a critical age (or axonal length) and then plateau. This could indicate that the deficits progress as a result of axonal lengthening in association with limb growth.

Human sensory axons are lengthy and can extend for one metre or more. It is known that many axonal proteins (including cytoskeletal components, organelles, mitochondria and synaptic vesicle precursors) must be transported along the axon in order to reach their targets [87]. This leads to the classic "distal first" theory of axonal neuropathies, which is thought to be the result of longer axons needing higher levels of axonal transport [4, 87, 135]. In fact, our observations could support one of two theories: either that sensory neurons in these patients can only produce and/or nurture axons of a certain maximal length, or that axonal pathologic processes that interfere with cell survival do so in a manner that is proportional to axonal length.

Table XIII

Epidemiologic Features of Québec HSAN2 Cohort

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Family	A	A	I	B	B	C	D	E	J	F	G	H	I	J	J
Origin	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan	FrCan
Consanguinity*	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-
Gender	M	F	F	F	F	F	M	M	F	M	F	M	F	F	F
Age at examination	0.7	4	12	19	22	25	28	31	33	35	43	44	58	71	73
No. of affected siblings/total	2/2	2/2	1/2	2/2	2/2	1/3	1/2	2/3	1/3	2/8	1/6	1/1	3/6	3/4	3/4
Apparent mode of transmission	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR

* = first degree relationship between parents; F = female; M = male; FrCan = French-Canadian; AR = autosomal recessive; + = present; - = absent

Note:

Case 7	Sister refused to participate in study
Case 10	Sister deceased at twenty years of age from septic shock secondary to infected ulcer
Case 13	One affected brother deceased; second brother not recruited
Cases 14 and 15	Sister deceased at seven years of age from infected ulcer

Table XIV

Historical Characteristics of Québec HSAN2 Cohort

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Average
Age at diagnosis (yrs.)	0.7	4	6	10	5	8	8	11	9	11	3	12	9	10	13	8
Age sensory deficit first noted	0.7	4	-	-	-	5	6	-	-	-	9	-	6	10	7	7
Age at first soft tissue infection	-	-	6	10	5	7	6	18	5	12	3	12	7	10	7	8
Site of infection	-	-	foot	toes	tibia	foot	foot	foot	foot	finger	foot	finger	toe	-	hand	-
Age at first fracture	-	4	9	12	5	10	8	12	9	10	14	13	8	10	13	10
Type of fracture	-	trauma	trauma	trauma	-	trauma	-	-	trauma	trauma	-	-	spontaneous	trauma	trauma	-
Age at first necrosis	-	-	10	16	-	-	14	20	16	16	7	10	11	10	13	13
Age at first amputation	-	-	-	16	-	-	18	27	16	22	-	-	10	10	13	17
Diplopia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deafness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lightning pains	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Postural hypotension	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sphincter disturbance	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alacrima	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = absent

Table XV

Clinical and Electrophysiologic Features of Québec HSAN2 Cohort

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age at examination (years)	0.7	4	12	19	22	25	28	31	33	35	43	44	58	71	73
Extraocular muscle function	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sensory trigeminal nerve function	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Deltoid muscle strength	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Biceps reflex	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-
Triceps reflex	+	-	+	-	-	+	-	-	-	-	+	+	-	-	-
Patellar reflex	+	-	-	-	-	-	-	-	-	-	-	-	N/A	-	-
Achilles' reflex	-	-	-	-	-	-	-	-	-	-	-	-	N/A	-	N/A
Ulnar SNAP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

N = normal; - = absent; + = present; SNAP = sensory nerve action potential; N/A = not available because amputated

Amputations and Necrosis of Extremities in Initial Québec HSAN2 Cohort

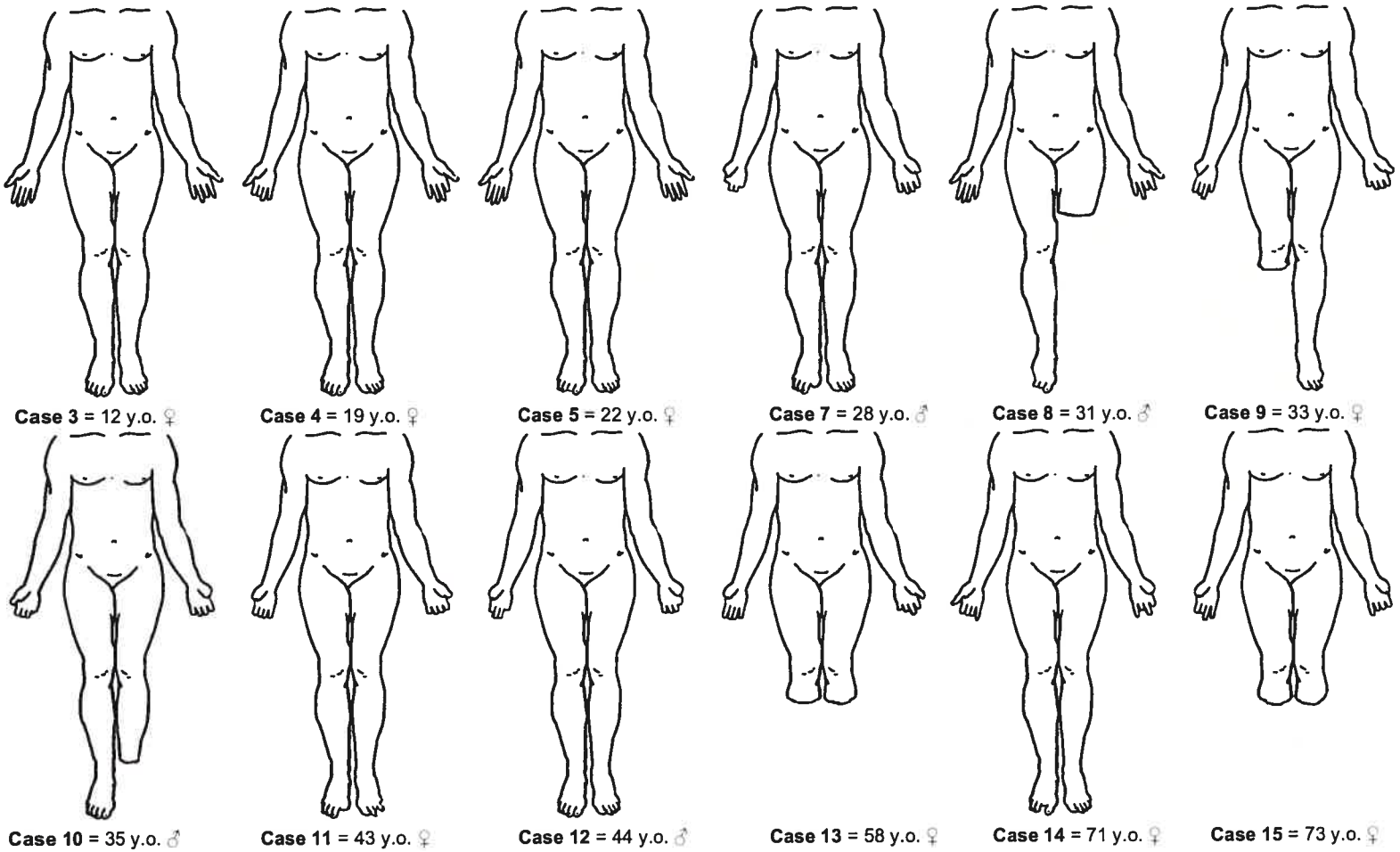

Figure 5. Amputations and Necrosis of Extremities in Québec HSAN2 Cohort. Cases one, two, and six, who were intact, are not shown. (Figure based on Melzack, 1975 [165])
y.o. = year-old; ♀ = female; ♂ = male.

Table XVI

Pathologic Features of Québec HSAN2 Cohort

Case	Biopsy	Findings
4	Sural nerve	-"compatible" with HSAN2 according to medical chart notes; official report not available
7	Sural nerve	-diffuse axonal atrophy and degeneration -almost complete demyelination -fibroblasts in endoneurium with few Schwann cells -increased endoneurial collagen -increased perineurial spaces -very large nuclei with nuclear inclusions in Schwann cells -lipid vacuoles in Schwann cells
	Muscle	-minor myofibrillar changes compatible with secondary changes
9	Sural nerve	-complete loss of myelinated fibers -unmyelinated fibers slightly increased, but normal
	Tibialis anterior	-same as sural nerve, but presence of some normal myelinated fibers
10	Sural nerve	-total loss of myelinated fibers -severe loss of unmyelinated fibers; normal appearance of residual fibers -reactional Schwann cell changes -no regenerative phenomena

TABLE XVII

Serial Neurologic Examinations Showing Deficit Progression in Québec HSAN2 Cohort

Case	4	5	7	8	9	10	13
Age at initial examination	10	5	6	5	11	15	9
DTR	-	+	↓	+	↓	-	-
Sensory deficit	to wrists, knees	glove-and-stocking	to fingers, feet	tact normal, pinprick decreased	to wrists, ankles	to elbow, distal 1/3 of thigh	periphery of four limbs
Age at first examination *demonstrating progression*	19	10	13	31	33	35	58
DTR	-	-	-	-	-	-	-
Sensory deficit	to elbows	mid-arm, mid-thigh	to hands, knees	to mid-forearm	to shoulder	to mid-arm	to shoulder

+ = present, - = absent,
↓ = decreased

Table XVIII

Levels of Anesthesia in Québec HSAN2 Cohort

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Average
Axon length of upper limb (cm)	-	54	70	74	70	71	82	87	76	81	83	85	76	75	71	75
Anesthesia to touch (cm)	-	-13	13	10	14	-4	21	7	14	20	17	52	17	26	15	15
Anesthesia to pinprick (cm)	-	-13	0	4	13	6	27	5	10	17	10	9	10	5	14	8
Residual tactile sensation (cm)	-	67	57	64	56	75	61	80	62	61	66	33	59	49	56	60
Residual pinprick sensation (cm)	-	67	70	70	57	65	55	82	66	64	73	76	66	70	57	67

Table XIX

Levels of Hypoesthesia in Québec HSAN2 Cohort

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Average
Axon length of upper limb (cm)	-	54	70	74	70	71	82	87	76	81	83	85	76	75	71	75
Hypoesthesia to touch (cm)	-	7	23	16	21	4	51	28	56	23	17	56	42	61	24	31
Hypoesthesia to pinprick (cm)	-	-7	18	13	29	10	36	7	23	27	10	13	58	32	24	21
Residual normal touch (cm)	-	47	47	58	49	67	31	59	20	58	66	29	34	14	47	45
Residual normal pinprick (cm)	-	61	52	61	41	61	46	80	53	54	73	72	18	43	47	54

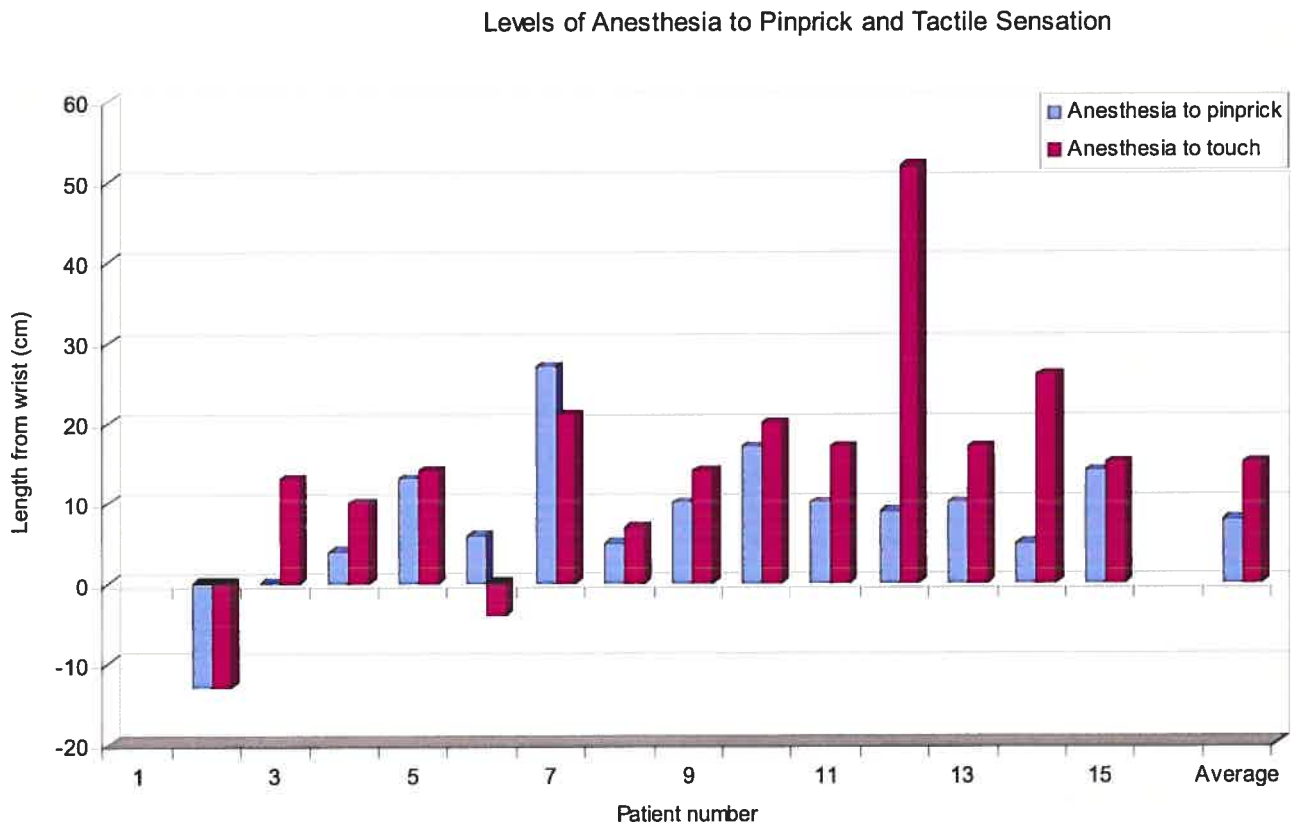

Figure 6. Levels of Anesthesia to Pinprick and Tactile Sensation. Anesthesia to tactile stimulation was greater than or equal to anesthesia to pinprick stimulation in all but two cases (numbers 6 and 7). Our convention of measurement from the wrist proximally (due to the presence of distal amputations in many patients), resulted in negative values for case number two, who had normal sensation to her fingertips for both modalities, and for case number six, who had normal tactile sensation below the wrist.

XXX

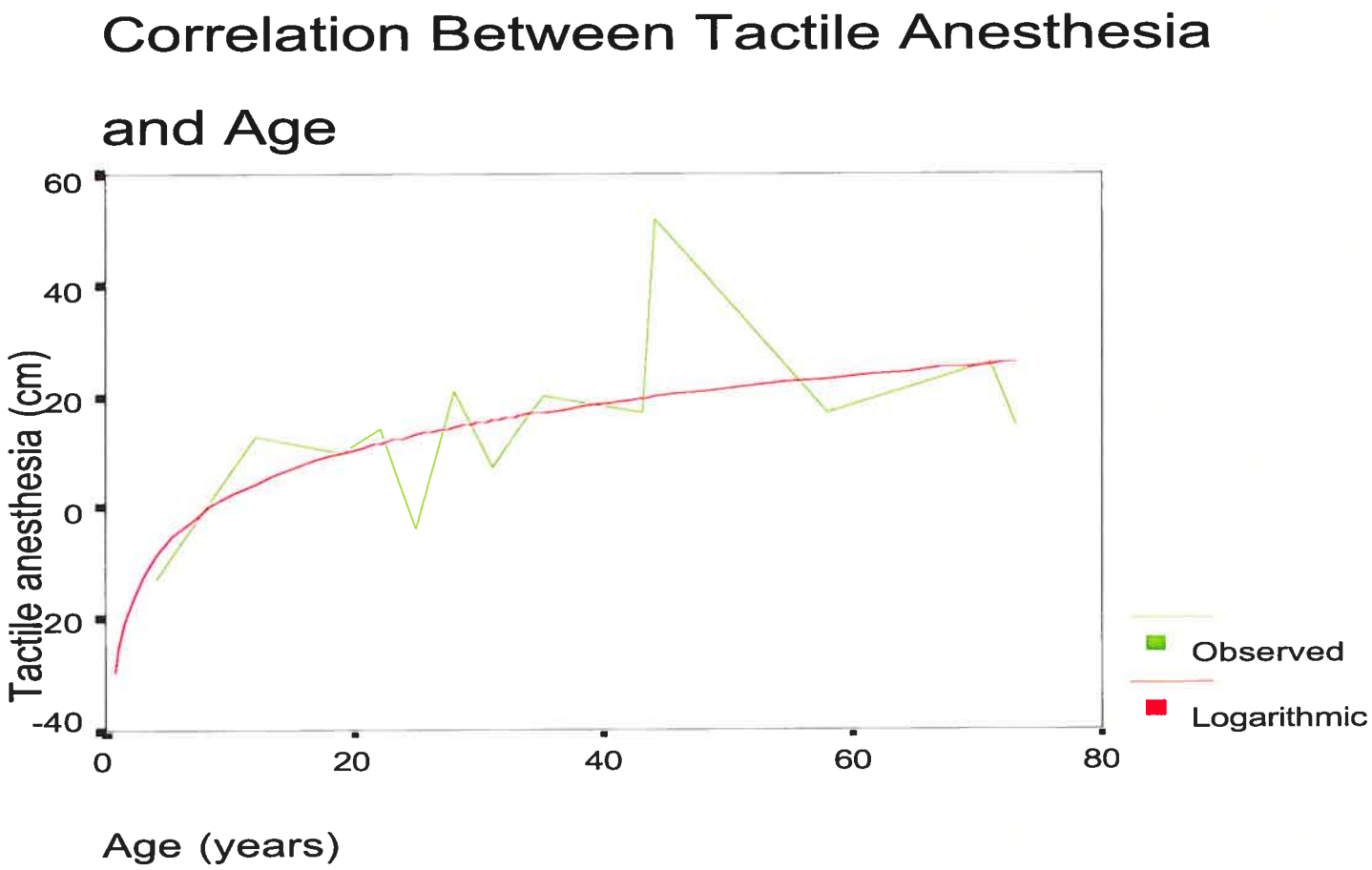

Figure 7. Correlation Between Tactile Anesthesia and Age. The logarithmic relationship between tactile anesthesia and age shows a tendency to plateau after the age of twenty years.

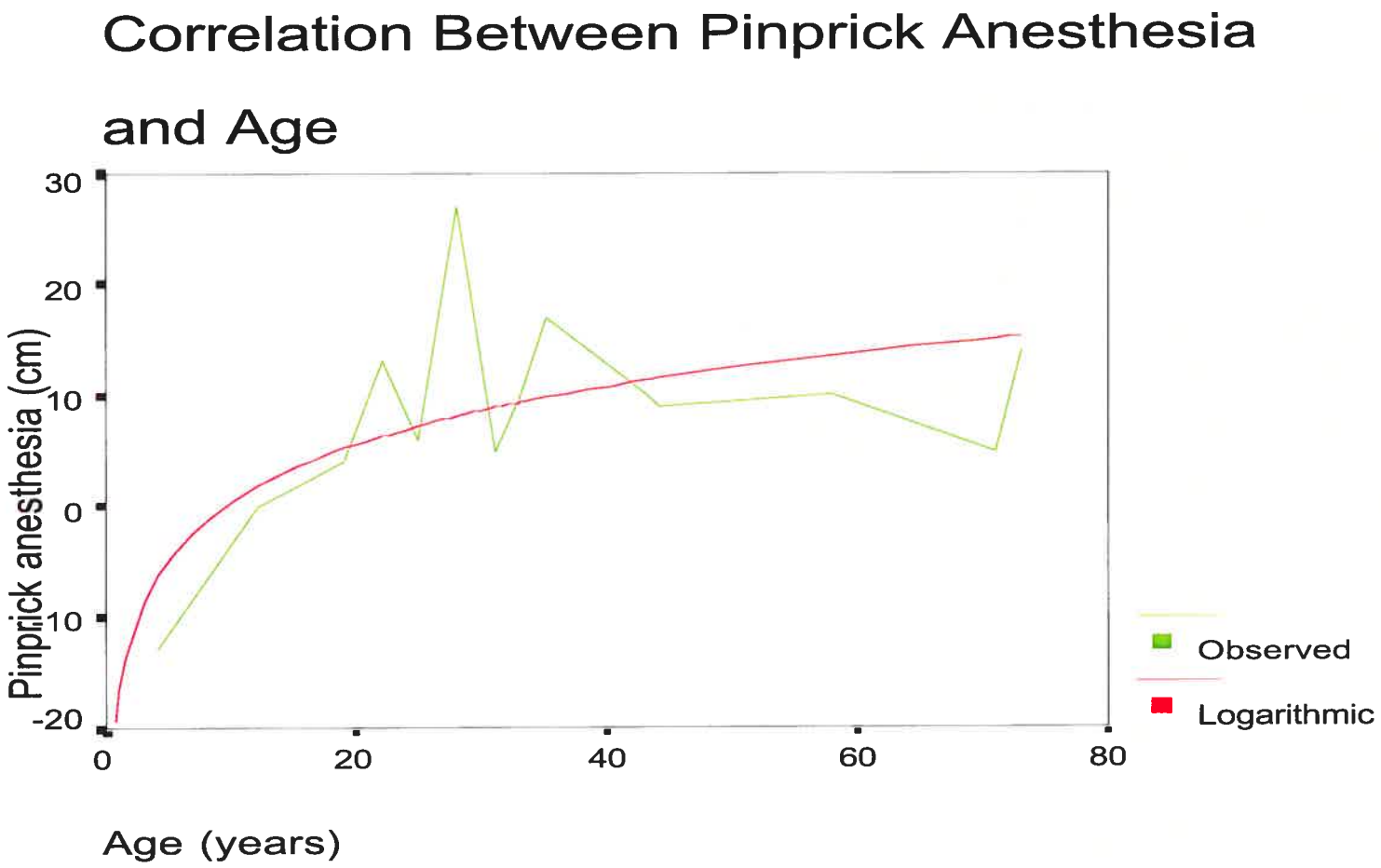

Figure 8. Correlation Between Pinprick Anesthesia and Age. The logarithmic relationship shows a tendency to plateau after the age of twenty years, as is the case for tactile stimulation.

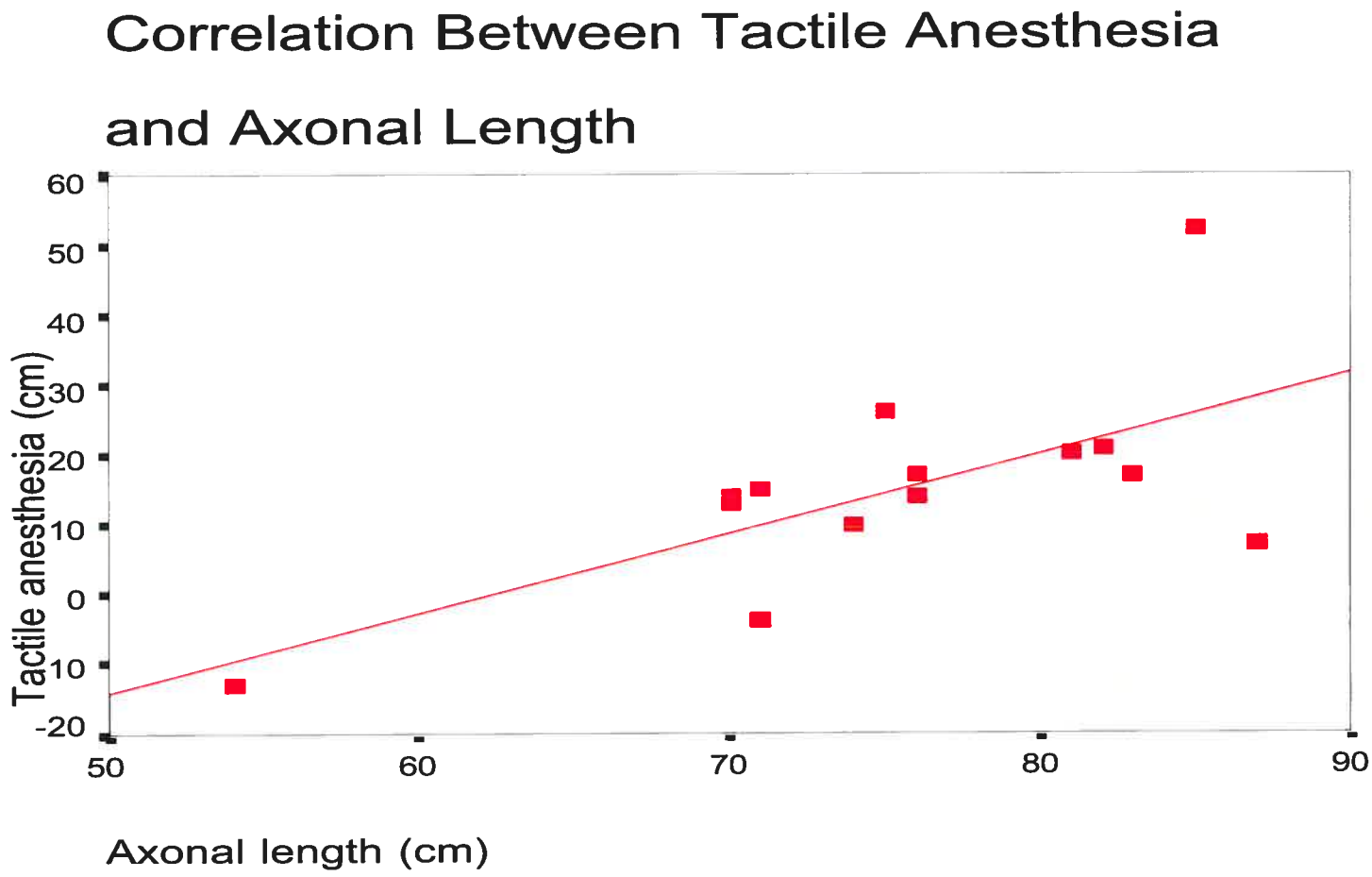

Figure 9. Correlation Between Tactile Anesthesia and Axonal Length. This linear relationship (p = 0.01) demonstrates that the sensory deficit is directly proportional to axonal (arm) length.

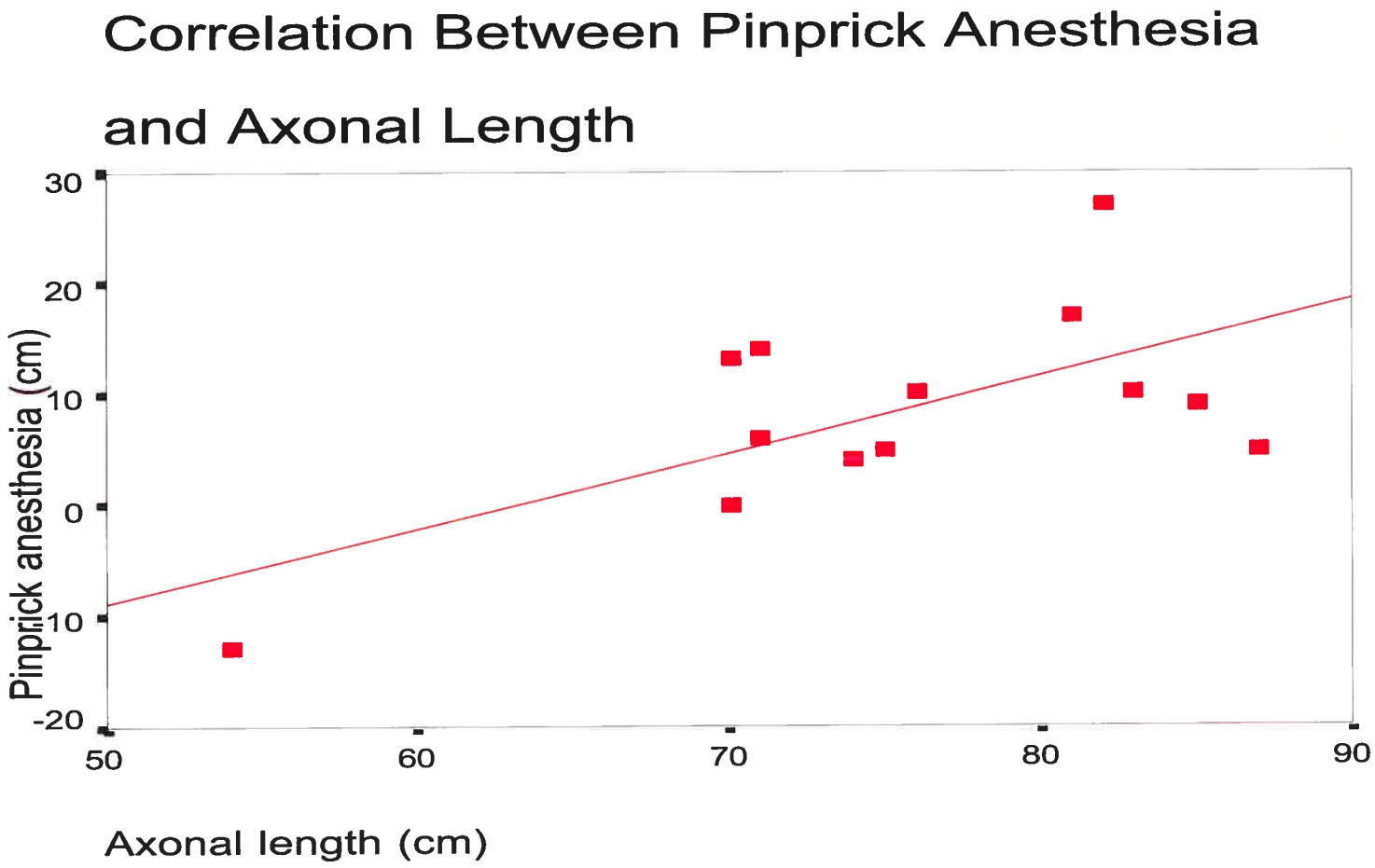

Figure 10. Correlation Between Pinprick Anesthesia and Axonal Length. This linear correlation (p = 0.02) demonstrates that the sensory deficit is directly proportional to axonal (arm) length.

Figure 11. Correlation Between Residual Pinprick Sensation and Age. This linear relationship with a slope approaching zero shows that the absolute length of normal sensation remains relatively constant with age. This correlation was not shown to be statistically significant.

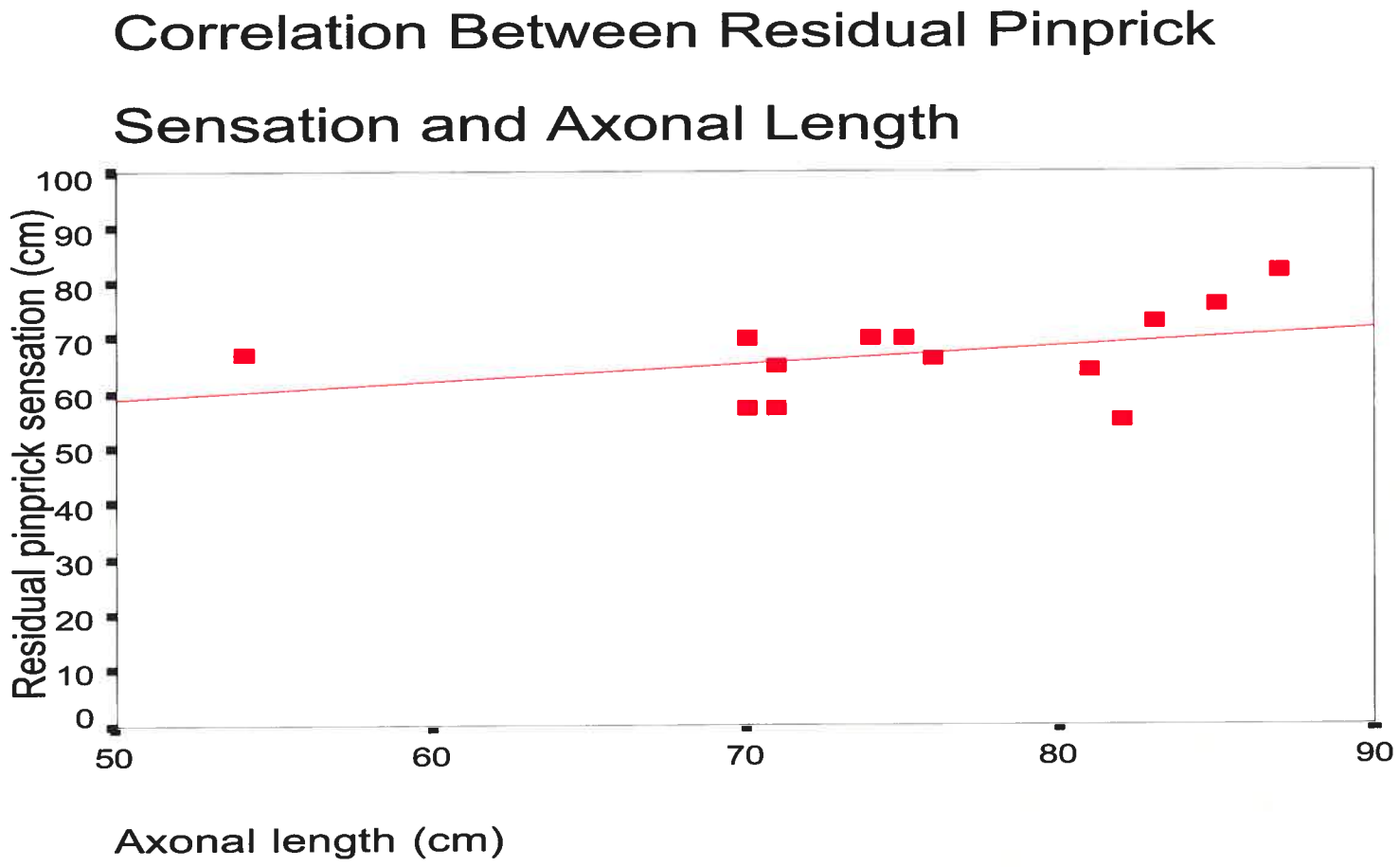

Figure 12. Correlation Between Residual Pinprick Sensation and Axonal Length. This linear relationship with a slope approaching zero demonstrates a tendency for absolute length of normal sensation to remain constant despite arm growth. This correlation was not shown to be statistically significant.

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

Article :

K. Roddier BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Sarrazin MD, A. Larbrisseau MD, M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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Tina Thomas

Programme de maîtrise en sciences neurologiques

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Bernard Brais [redacted] 2007-06-14

Coauteur Date

Marie-Josée Dicaire [redacted] 2007-06-14

Coauteur Date

Coauteur Signature Date

Coauteur Signature Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

Article :

K. Roddier BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Sarrazin MD, A. Larbrisseau MD, M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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Coauteur	Signature	Date
ANNE-MARIE GAGNON	[redacted]	21/06/07

Coauteur Signature Date

Coauteur Signature Date

Coauteur Signature Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

Article :

K. Roddier BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Sarrazin MD, A. Larbrisseau MD, M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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Coauteur	Signature	Date
Daniel Gaudet	[redacted]	7/06/07

Coauteur Signature Date

Coauteur Signature Date

Coauteur Signature Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

Article :

K. Roddier BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Sarrazin MD, A. Larbrisseau MD, M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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Coauteur	Signature	Date
Isabelle Gosselin	[redacted]	18/06/2007
Coauteur	Signature	Date
Coauteur	Signature	Date
Coauteur	Signature	Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

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Coauteur	Signature	Date
MARIE Lambert	[redacted]	18-06-2007

Coauteur Signature Date

Coauteur Signature Date

Coauteur Signature Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

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Coauteur	Signature	Date
A. LARBRISSEAU	[redacted]	20/06/07

Coauteur Signature Date

Coauteur Signature Date

Coauteur Signature Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

Article :

K. Rodilon BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Carrazco MD, A. Larbrisseau MD, M. Larbert MD, M. Vanasse MD, D. Caccet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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Geneviève Marleau	[redacted]	21 juin 2007
Coauteur		Date
Coauteur	Signature	Date
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ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

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K. Roddier BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Sarrazin MD, A. Larbrisseau MD, M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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Katel Roddier	[redacted]	01-Jul-2007
Coauteur		Date

Coauteur	Signature	Date
Coauteur	Signature	Date
Coauteur	Signature	Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

Article :

K. Roddier BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Sarrazin MD, A. Larbrisseau MD, M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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GUY-A ROULEAU [redacted] 19/06/2007

Coauteur Signature Date

Coauteur Signature Date

Coauteur Signature Date

Coauteur Signature Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

Article :

K Roddier BSc, T. Thomas MD, G. Marleau BSc, A M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc. I. Gosselin BSc A.M. Sarrazin MD, A. Larbrisseau MD. M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, C.A Rouleau MD Ph.D., B. Brais MD PhD

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Coauteur	Signature	Date
SARRAZIN Anne-Marie	[redacted]	2007/06/19
Coauteur	Signature	Date
Coauteur	Signature	Date
Coauteur	Signature	Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

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K. Roddier BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Sarrazin MD, A. Larbrisseau MD, M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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Coauteur	Signature	Date
Anik St-Denis	[redacted]	18-06-07

Coauteur Signature Date

Coauteur Signature Date

Coauteur Signature Date

ACCORD DES COAUTEURS

Tina Thomas

Programme de maîtrise en sciences neurologiques

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K. Roddier BSc, T. Thomas MD, G. Marleau BSc, A.M. Gagnon BSc, M.J. Dicaire BSc, A. St-Denis MSc, I. Gosselin BSc, A.M. Sarrazin MD, A. Larbrisseau MD, M. Lambert MD, M. Vanasse MD, D. Gaudet MD PhD, G.A. Rouleau MD Ph.D., B. Brais MD PhD

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Coauteur	Signature	Date
MICHEL VANASSE	[redacted]	07-06-19

Coauteur Signature Date

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The student, Tina Thomas, is authorized to include the aforementioned article in her master's thesis entitled "A Québec Mystery Unveiled: The Quest to Understand Hereditary Sensory and Autonomic Neuropathy Type 2."

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TINA THOMAS
[redacted]
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Accord des étudiants et du directeur de recherche
pour le partage d'un article entre deux mémoires de maîtrise

Je soussigné, Tina Thomas, donne mon accord pour que l'article « Two mutations in the *HSN2* gene explain the high prevalence of HSAN2 in French Canadians » qui a été accepté pour publication le 25 janvier 2005 dans la revue *Neurology* soit inclus dans le mémoire de Mme Katel Roddier (Maîtrise en Biologie moléculaire, faculté des Études supérieures) intitulé « Identification des mutations responsables de la NHSA2 et de l'AOA2 dans la population canadienne-française : Deux nouveaux exemples de maladies à effet fondateur au Québec »: ainsi que dans mon mémoire.

[redacted]
Dre Tina Thomas

19 oct 2005
Date

Je soussignée, Katel Roddier, donne mon accord pour que l'article « Two mutations in the *HSN2* gene explain the high prevalence of HSAN2 in French Canadians » qui a été accepté pour publication le 25 janvier 2005 dans la revue *Neurology* soit inclus dans le mémoire de Dre Tina Thomas (Maîtrise en Sciences neurologiques, faculté de médecine) intitulé : « A Québec Mystery Unveiled: The Quest to Understand Hereditary Sensory and Autonomic Neuropathy Type 2» ainsi que dans mon mémoire.

[redacted]
Mme Katel Roddier

09/11/05
Date

Je soussigné, Bernard Brais, donne mon accord pour que l'article « Two mutations in the *HSN2* gene explain the high prevalence of HSAN2 in French Canadians » qui a été accepté pour publication le 25 janvier 2005 dans la revue *Neurology* soit inclus dans les mémoires de Dre Tina Thomas (Maîtrise en Sciences neurologiques, faculté de médecine) et de Mme Katel Roddier (Maîtrise en Biologie moléculaire, faculté des Études supérieures).

[redacted]
Dr Bernard Brais

05-11-09
Date